Thematic area: Master thesis in Analytical Petroleum Chemistry and Renewable Biofuels

Direct Injection Electrospray Mass Spectrometry (DI-ESI-MS) and Liquid Chromatography Electrospray Mass Spectrometry (LC-ESI-MS) method development for characterisation of organic acids in petroleum and bio-oil samples

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Thesis for the degree of

Master of Science in Chemistry

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March 2017

"Analytical method development is like draughts. It matters less how many times you made wrong moves, you only need to be right once". **~R. O. Abia**

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Romanus O. Abia
Bergen, 2017.

TABLE OF CONTENTS

LIST OFFIGURES

LIST OF TABLES

LIST OF ABRREVIATIONS

ACN: Acetonitrile

APCI: Atmospheric pressure chemical ionisation

DCM: Dichloromethane

DHB: Dihydrobenzene

DHBA: Dihydrobenzoic acid

DI: Direct injection/infusion

DI-ESI-MS: Direct injection electrospray ionisation mass spectrometry

EIC: Extracted ion chromatogram

ESI: Electrospray ionisation

EU: European Union

FT-ICR-MS: Fourier transformer ion cyclotron resonance mass spectrometry

GC-MS: Gas chromatography mass spectrometry

HPLC: High performance liquid chromatography

LC: Liquid chromatography

LC-MS/MS: Liquid chromatography tandem mass spectrometry

LCMs: Lignocellulosic materials

LC-ESI-MS: Liquid chromatography electrospray mass spectrometry

MeOH: Methanol

MS: Mass spectrometry

RP: Reverse phase

SIM: Selected ion monitoring

SRM: Selected reaction monitoring

TIC: Total ion current

TOF: Time-of-flight

Keywords: *Petroleum acids, lignin-derived biofuels, petroleum hydrocarbons, complex mixtures, polyaromatic compounds, petroleum biomarkers, chemical characterisation, chemical fingerprinting, fractionation, biodegradation, liquid chromatography – mass spectrometry, electrospray ionisation, data analysis.*

ABSTRACT

Analysis and characterisation of complex mixtures such as petroleum and bio-oils are unarguably challenging. Finding suitable analytical method therefore needs to be considered carefully by analytical chemists. Electrospray Ionisation (ESI) can be used to give a profile of the organic molecules in the mixture.

A liquid chromatography electrospray ionisation mass spectrometry method for the separation and profiling of organic compounds in crude oil and lignin derived bio-oil mixtures has been developed. The analytical method was developed in a reverse phase chromatography with gradient elution program using water and acetonitrile plus 0.1% acetic acid. The method was developed using standard compounds which include, naphthoic acid, naphthol, methyl benzoic acid, guaiacol, guaiacol glyceryl ether, catecol, dihydroxybenzene, dihydroxybenzoic acid, methyl catecol, ethyl catecol, quinolinol, NH_2-benzamide, and carbazole. The separation is reproducible over repeated injection of the standard compounds with an acceptable variation in the retention times.

A solid phase extraction protocol using a normal phase cyano-bonded stationary phase was modified to fractionate the petroleum and bio-oils into polar and non-polar components. Hexane/dichloromethane was used to elute the non-polar and hydrocarbon components of the bio-oil and petroleum respectively, while acetonitrile and methanol was used to elute the polar fractions which were submitted to the reverse phase column for separation and profiling.

The application of the DI-ESI and LC-ESI-MS method both in direct fingerprinting and polar fractions of the both oils showed that molecular masses of interest were detected. These include, *m/z* 121 (benzoic acid), *m/z* 143 (naphthol) and *m/z* 171 (naphthoic acid) for crude oils. In bio-oils, m/z 109 (catecol or dihydroxybenzene), *m/z* 137 (ethyl catecol), m/z 143 (naphthol), *m/z* 123 (methyl catecol or guaiacol), *m/z* 151 (propylbenzene-1,3-diol), *m/z* 165 (propyl guaiacol), *m/z* 177 (methoxy eugenol), *m/z* 179 (guaiacol acetone) and 175, 191, 193, 199, 243, 285 etc., were detected.

1.0 INTRODUCTION

1.1 Petroleum and Biofuels

Analysis and characterisation of complex mixtures such as petroleum and bio-oils are unarguably challenging. Finding suitable analytical method therefore needs to be considered carefully by analytical chemists. Electrospray Ionisation (ESI) can be used to give a profile of the organic molecules in the mixture. The profile of the components in the crude oil samples are very complex, and multivariate methods are very useful to interpret the data and identify series of homologous compounds with the same basic structures. Previous work in our group has established a procedure for the data handling for bio-oils. The same procedure can be used in later stage for the crude oil samples. This project aims to develop analytical method to identify and characterise bio-surfactants in crude oils including biodegraded oils as well as polar compounds in lignin derived bio-oils. Firstly, a fingerprinting approach will be used on the crude petroleum and the bio-oils, afterwards the bulk oil will be fractionated to isolate non-hydrocarbons-including surfactants, and the relevant fractions will be analysed by LC-ESI-MS and/or DI-ESI-MS. In-depth study of the complex spectra may later be analysed using internally developed software in our research group.

Petroleum, also known as crude oil is the main source of energy for industries and daily life. It is the product of natural changes of organic debris over millennia. Crude oil is formed by alteration and decomposition in millions of years of biomass materials, mostly aquatic materials. When these organisms die, they decompose, and the remains sink into the sea floors, and get buried as sedimentary rocks. The end point of the complicated process is the formation of a compound mixture, called crude oil, which comprises a number of different compounds [1, 2] . Details of petroleum constituents are discussed in section 2.1.

Biofuel is loosely, a fuel derived directly from organic matter. They are liquid or gaseous transport fuels such as biodiesel and bioethanol which are basically made from biomass. They serve as a renewable alternative to fossil fuels around the world, helping to reduce greenhouse gas emissions. By 2020, the European Union (EU) aims to have 10% of the transport fuel of every EU country come from renewable sources such as biofuels. Fuel suppliers are also required to reduce the greenhouse gas intensity of the EU fuel mix by 6% by 2020 in comparison to 2010.

The two most common types of biofuels in use today are ethanol and biodiesel. Ethanol is an alcohol, the same as in beer and wine (although ethanol used as a fuel is modified with additives to make it undrinkable). It is most commonly made by fermenting any biomass high in carbohydrates through a process analogous to beer brewing. However, the subject matter is beyond the scope of the present work which is focused on the characterisation of acids in complex mixtures. Such acids in the case of bio oils are products of lignin to liquids (LtL) conversion. The reader who wants more study on bioethanol productions should consult [3-6] for more comprehensive study on the subject matter. Further details of biofuel and its constituents are discussed in section 2.2.

1.2 Project objectives

1. Develop ESI-MS method (s) that can ionise and detect O and N-containing organic compounds in complex mixtures.
2. Develop a suitable HPLC procedure for separating O and N-containing organic compounds which is suitable for LC-MS by ESI.
3. Develop a suitable fractionation procedure for the preparation of samples for LC-ESI-MS and DI-ESI-MS.
4. Apply the ESI-MS method in direct fingerprinting of petroleum and lignin based bio-oils.
5. To test the fractionation and LC-MS methods for characterisation of fractionated petroleum and lignin based bio-oils.

2.0 BACKGROUND

2.1 Petroleum

Petroleum is a complex mixture and may be grouped into four compound classes: the saturates, the aromatics, acidic and basic resins. The acids may include: phenols, fatty acids, ketones, esters, and porphyrins, while the bases include: pyridines, quinolines, carbazoles, sulfoxides, and amides. In addition, there are number of ways to classify petroleum fractions. The more general way of doing this is to group the crude oils into saturated hydrocarbons which could be branched or straight chains. These group are generally referred to as *paraffins* (alkanes and cycloalkanes). Another group falls into the cyclic saturates which may have varying degrees of paraffinic side chains and are mostly referred to as alicyclics, these group are generally called *naphthenes* [1, 7].

Furthermore, *aromatic* hydrocarbons are group of petroleum components having varying aromatic backbones that can be linked to naphthenes and/or paraffinic side chains. Components of petroleum which contain heteroatoms are called *resins and asphaltenes*. These group of petroleum consists of polar compounds, containing one or more sulphur, nitrogen and/or oxygen atoms. Petroleum are generally grouped as SARA fractions (Saturates, Aromatics, Resin and Asphaltenes) [1, 7, 8].

Alternatively, crude oils are classified as heavy or light oils according their density or API gravity and how they perform during the distillation of the crude into several components. Light Crude oil is liquid petroleum that has low density and that flows freely at room temperature, light oils have API gravities greater than 40°, they have high contents of paraffins which gives them low boiling points. In contrast to the above definition heavy oils are those with density or specific gravity higher than that of light crude oil. Heavy crude oil may be referred to as liquid petroleum with an API gravity less than 20°and they are rich in asphaltenes, with extra heavy oils having an API gravity between 5-10° [6]. Furthermore, oils having API gravities between 20 and 40° are classified as intermediate oils. In a light crude, the contents of true hydrocarbon compounds could be up to 97% while this could go down to 50% in heavy oils [6]. Heavy oils are characterised by high boiling points, high viscosity and probably high sulphur contents, while light oils are complementary. The heavy crude often contains high

contents of non-hydrocarbons such as naphthenic acids and compounds classified as petroleum acids.

Moreover, over millions of years in the petroleum reservoir, a number of microbes can consume hydrocarbons by producing surfactants that help them access the petroleum hydrocarbons they use as a carbon sources [9]. These surfactants influence the properties of oils in different ways, depending on the type of bacteria and type of surfactants produced. The exact surfactants found in innately degraded crude oils are still subject of investigation and to this day mostly unknown [9].

As can be seen above, the number of chemical compounds in crude oils is enormous and thus confers its characteristic complexity. The molecular characterisation of the components of crude oil is almost impossible due to the extreme complexities of the chemical compounds ubiquitous to petroleum [10]. However, chemical fingerprinting of the crude oil components can be achieved. Fractionation is a step in molecular characterisation of crude oils into various components with similar chemical properties.

2.1.1 N- and S-containing molecular types in petroleum

Petroleum being a complex mixture is made up of paraffin, naphthenes and aromatic hydrocarbons and other NSO (nitrogen, sulphur and oxygen) compounds. The nitrogen compounds are generally grouped into two, the basic and non-basic groups each of which may have alkyl side chains and other ring systems, Figure 2.1 outlines the subdivision of organonitrogen compounds in petroleum.

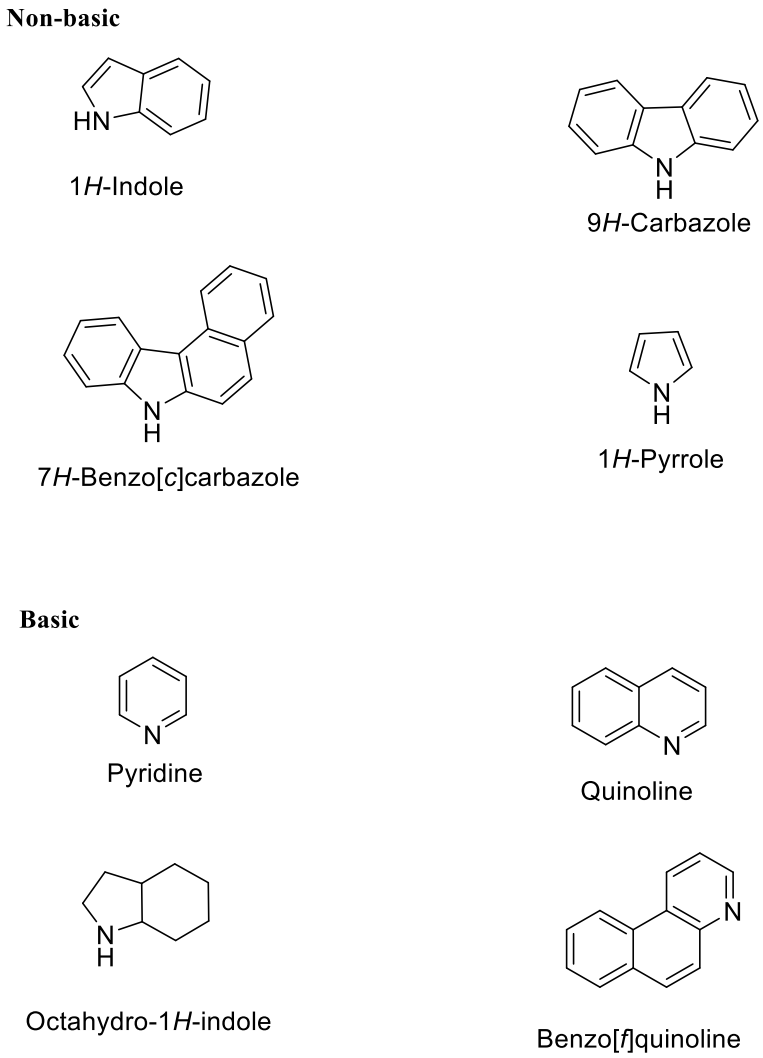


Figure 2.1: Subdivision of organonitrogen compounds of petroleum as basic and non-basic, adapted from [1].

The other group or type of nitrogen containing compounds in petroleum are the porphyrins, they consist of basically four pyrrole rings linked together by methylene groups at the carbon next to nitrogen atoms. Others are the oxygenate groups like phenols, naphthenic acids and esters. Just as the sulphur, nitrogen compounds, increases in quantity as the molecular mass of petroleum increases.

Different types of sulphur compounds occur in crude oils (Figure 2.2) which include mercaptans (-SH), the disulphides (-S-S-) and the sulphides (-S-). They are thermally unstable when compared to thiophenes, thus they tend to readily react to form more stable sulphur compounds. During chromatographic separation, these sulphur compounds are found in polar aromatics, even when they may not be entirely aromatics [2].

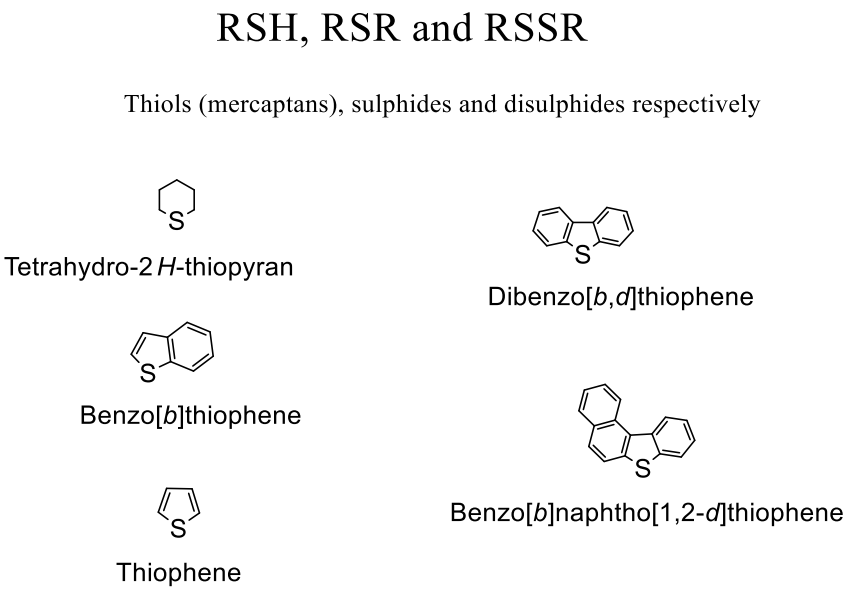


Figure 2.2: Types of sulphur containing compounds in petroleum adapted from [2].

2.1.2 Sources of petroleum acids

Biodegradation of petroleum compounds produces petroleum acids [11]; this process is carried by petroleum degrading microorganisms. The biodegradation of petroleum affects the chemistry and properties of petroleum. Biodegradation of petroleum hydrocarbons is a time-consuming process which is dependent on several factors such as the type and the amount of the hydrocarbons available for degradation [9]. The efficiency of microbial alteration of petroleum compounds vary to a great extent, it thus can be ranked as follows: linear alkanes >

branched alkanes > aromatics > cyclic alkanes [12]. Several factors influence the degradation of petroleum compounds [13], and the degradation process markedly affects the chemistry and thus changes the physical properties of crude oil.

Naphthenic acid (NA) is a term often used to describe unspecific mixture of several cyclopentyl and cyclohexyl carboxylic acids with molecular weights ranging from hundreds to several hundreds of atomic mass units. They are partially uncharacterised complex mixtures of carboxylic acids [14], resulting from the microbial oxidation of petroleum hydrocarbons. They are associated with the fouling of pipelines and process equipment in oil production and with corrosion in oil refineries. As by-products of the rapidly expanding oil (tar) sands industries, NAs are also pollutants and have proved to be toxic to a range of organisms. They also have important beneficial uses as fungicides, tyre additives and, paradoxically, also in the manufacture of corrosion inhibitors. Typical structures of simple naphthenic acids are shown in Figure 2.3.

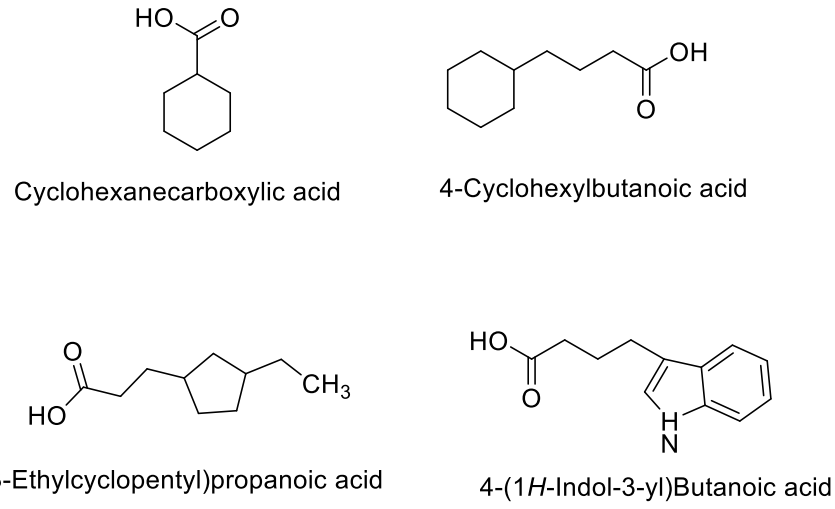


Figure 2.3: Selected typical structures of naphthenic acids.
Source: The structures of naphthenic acids were adapted from, https://en.wikipedia.org/wiki/Naphthenic_acid

These features make the characterisation of NAs an important goal for analytical chemists. The structural elucidation of petroleum acids has been a challenge facing chemists for over the years resulting largely from the extreme complexity of the mixtures. Naphthenic acids, are found in both crude oils and bitumen. The motivation for the interests in formation of petroleum and the requirements for maintaining crude oil quality and flow sparked early research into the structure of the naphthenic acids. This advancement lead to the understanding that NA also

play a great role in corrosion processes, and in the commercial use of naphthenic derivatives as wood preservatives and biocides [14, 15]. There are other forms of organic acids and other heterocyclic organic compounds such as quinolinol as can be seen in Figure 2.4.

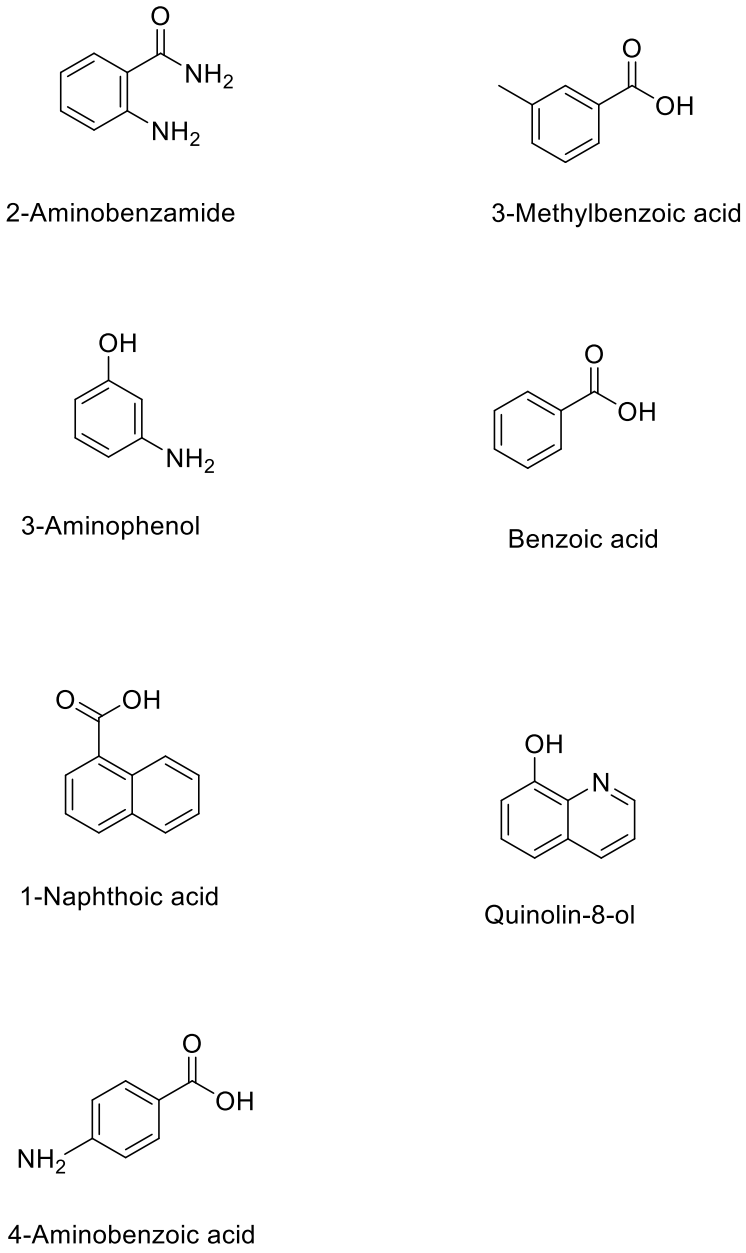


Figure 2.4: Structures of examples of selected organic compounds.
Source: The structures of organic acids were adapted from https://en.wikipedia.org and redrawn in ChemDraw.

2.2 Bio-oil

Many studies suggest that the supply of conventional oils will fall below the present consumption at around 20 years from now. The deficit in energy might ultimately be made by advancement in solar technologies, nuclear fusion etc., however the substitute for gasoline and diesel in internal combustion engines powered vehicles remains an onerous. Cellulose in the lignocellulosic biomass could be developed as source by many organic and bioorganic chemists in the task of harnessing the power of nature to ameliorate the scarcity of the plethora of chemicals that are products of conventional crude oils [5]. The drive and interest in search of alternative energy that offers, clean, renewable and sustainable energy in the world energy demand, the use of lignocellulosic materials (LCMs) for bioethanol production arises as a valuable substitute in this drive [16]. The first reported modern experiment with biofuels date back to 1973 in Brazil. The conventional oil scarcity in the year under reference, prompted the research in the use of glucose which is present as a sucrose in cane sugar to produce ethanol. The government of brazil offered tax advantages to those who would power their cars with ethanol as fuel component; by late 80s a high percentage of cars in Brazil could use ethanol [5]. However, ethanol as a volatile compound used as a substitute for gasoline has a drawback. Internal combustion engines burn fuels; ethanol, in comparison with typical hydrocarbon derived oils, contains more oxygen and thus generates less energy compared to hydrocarbon of a typical gasoline during combustion.

2.2.1 Lignocellulose

Lignocellulose is a biomass which is composed of carbohydrate polymers (cellulose, hemicellulose), and an aromatic polymer (lignin) consequently forming a three-dimensional polymeric composite called lignocellulose. Lignocellulosic biomass represents the vast bulk of land-based plant materials which include wood, grasses, agricultural and forestry residues [3-5]. Figure 2.5 outlines the process of conversion of lignocellulosic biomaterials to bio-oils and other high value end products.

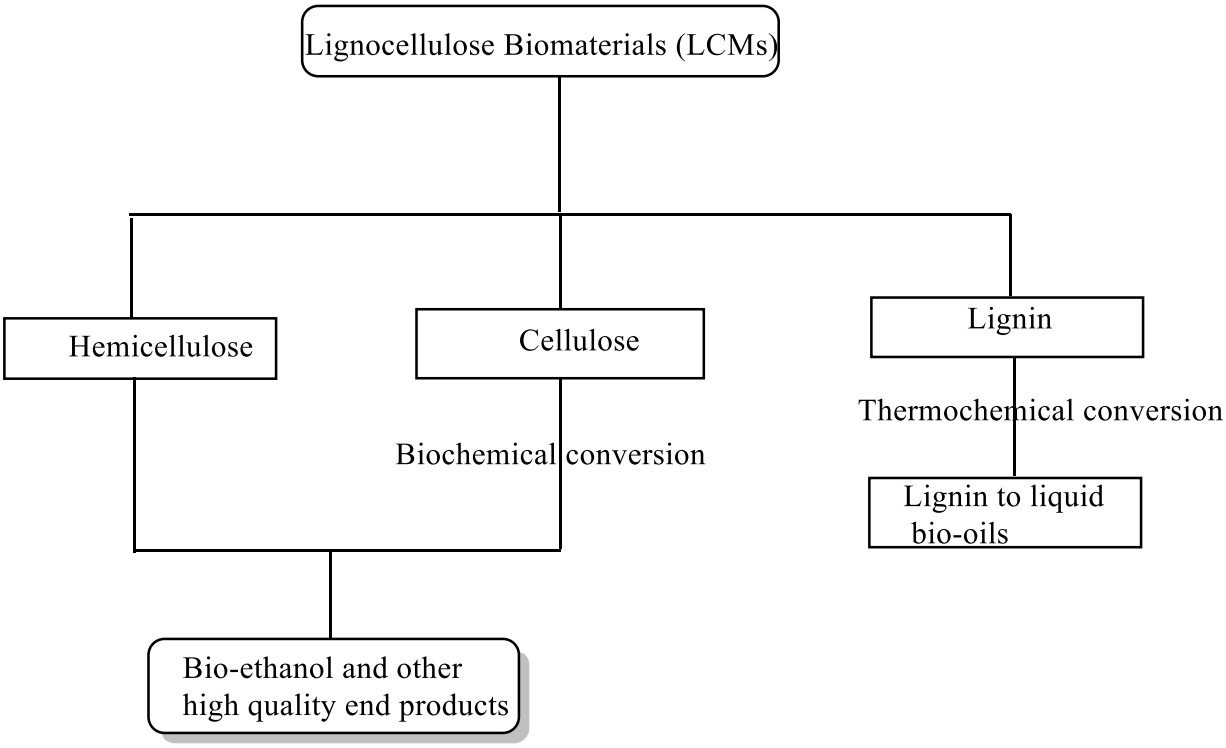


Figure 2.5: shows how lignocellulosic biomass can be converted to bio-oil.

2.2.1.1 Cellulose and Hemicellulose

Cellulose is a polysaccharide having the generic formula $(C_6H_{10}O_6)n$ yielding individual glucose monomers on hydrolysis. Cellulose is a ubiquitous carbohydrate in plants, it is a linear polymer of β-glucose, unbranched and almost like amylose but the type of configuration. Majority of cellulose production are used in paper manufacturing by wood-pulping operations and other industrial applications. Wood contains almost half of cellulose by mass, and a good number of textile fibres are derivatives of cellulose. Other derivatives of cellulose such as the cellulose acetate is an industrially important ester of cellulose and its useful in the wide range of products including cigarette filters, films and other coating materials. The usefulness of cellulose could also be found in the its application in pharmaceutical industries where it is used as matrices for the administration of slow release steroid drugs, coating of cosmetics, other pharmaceutical products and in moulding articles such as spectacle frames. Esters of cellulose are widely applied and used as drilling aids and in mining industries as well as detergents [3, 5, 6].

Hemicellulose is a vital component of plant cell walls which is made up to almost a third of woody plants tissues. It is a branched polymer that may comprise of many monomeric units of different sugar such as hexoses, pentoses with xylose in the highest percentage, forming a mixture of straight and highly branched chains of both C_5 and C_6 sugars. Their hydrolysis produces the C_6 sugars: glucose, mannose and galactose and the C_5 sugars: xylose and arabinose. C_5 hemicelluloses ($(C_5H_8O_4)n$) include xylan, arabinan and mannan, and they can occur in large amounts (20 to 40%) in corn cobs and corn stalks, and straws [5, 6]. The in-depth detail study on this is beyond the scope of the present work. For more detailed study on the cellulose and hemicellulose portion of lignocellulose biomaterial, the reader is advised to consult references [3-5].

2.2.1.2 Lignin

The lignin fraction is a 3-dimensional amorphous complex phenolic polymer. It is the third most abundant structural biopolymer and it is found in plant cell walls. The dominant monomeric units in the lignin polymers are benzene rings bearing methoxyl, hydroxyl and propyl groups that can be attached to other units. Lignin serves as the cement for the two other components of lignocellulose (cellulose and hemicellulose). It shields these other components from enzymatic and chemical degradation. It is the main non-carbohydrate polymer found in plants and it comprises of highly complex aromatic, and polyphenolic compounds, see Figure 2.6 for the complex lignin structure and Figure 2.7 for the lignin monomeric units (monolignols). Lignin may thus be defined as an amorphous, polyphenolic material arising from an enzyme-mediated dehydrogenative polymerization of three phenylpropanoid monomers, coniferyl, synapyl and *p*-coumaryl alcohols [3, 5, 6], as shown in Figure 2.7.

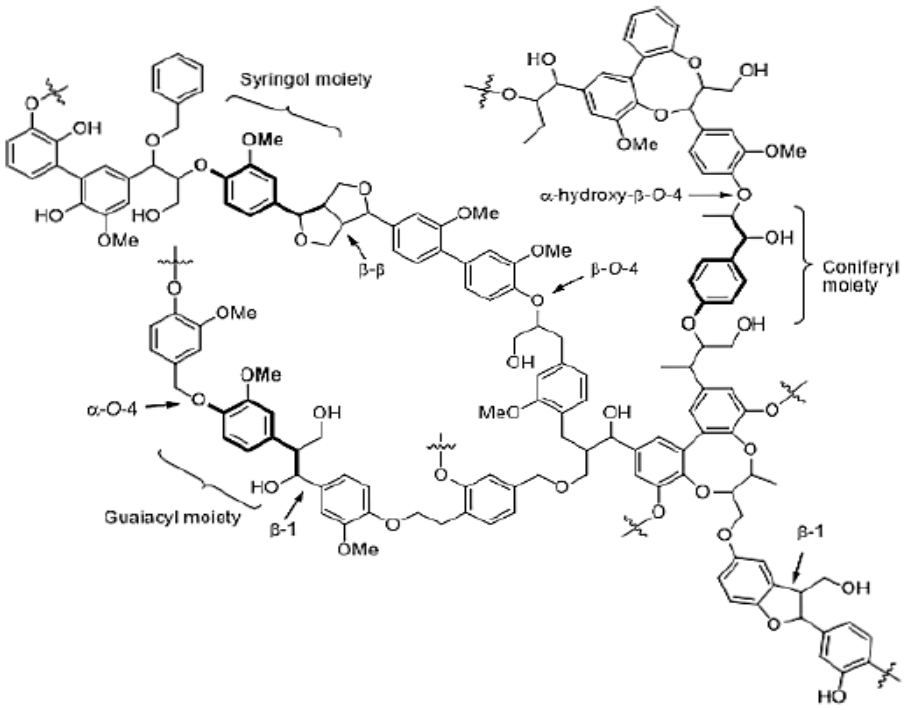


Figure 2.6: Example of possible lignin structure, a schematic representation of a typical softwood lignin macro structure [17].

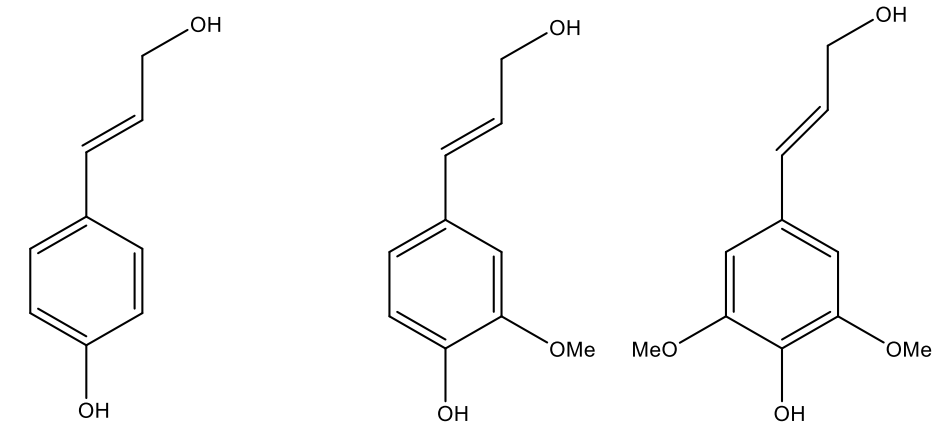


Figure 2.7: Structures of the three constituents of lignin adapted from [3].

2.2.1.3 Sources of acids in lignin based bio-fuels

In our group, much research has been carried out involving lignocellulosic biomaterials, focusing on thermochemical conversion of the lignin fraction of the fuels to bio-oils [17-22]. Different depolymerisation techniques have been employed such as the use of water, formic, methanol or ethanol as reaction media to study the significant impact on the bio-oil yields and chemical compositions [21]. On depolymerisation of the lignin fractions several acids and plethora of compounds are generated such as guaiacol, catechol, nahpthol, syringol etc. as well as the methylated and ethylated version of these compounds. Figure 2.8 outlines some selected compounds which can be derived from lignin depolymerisation processes.

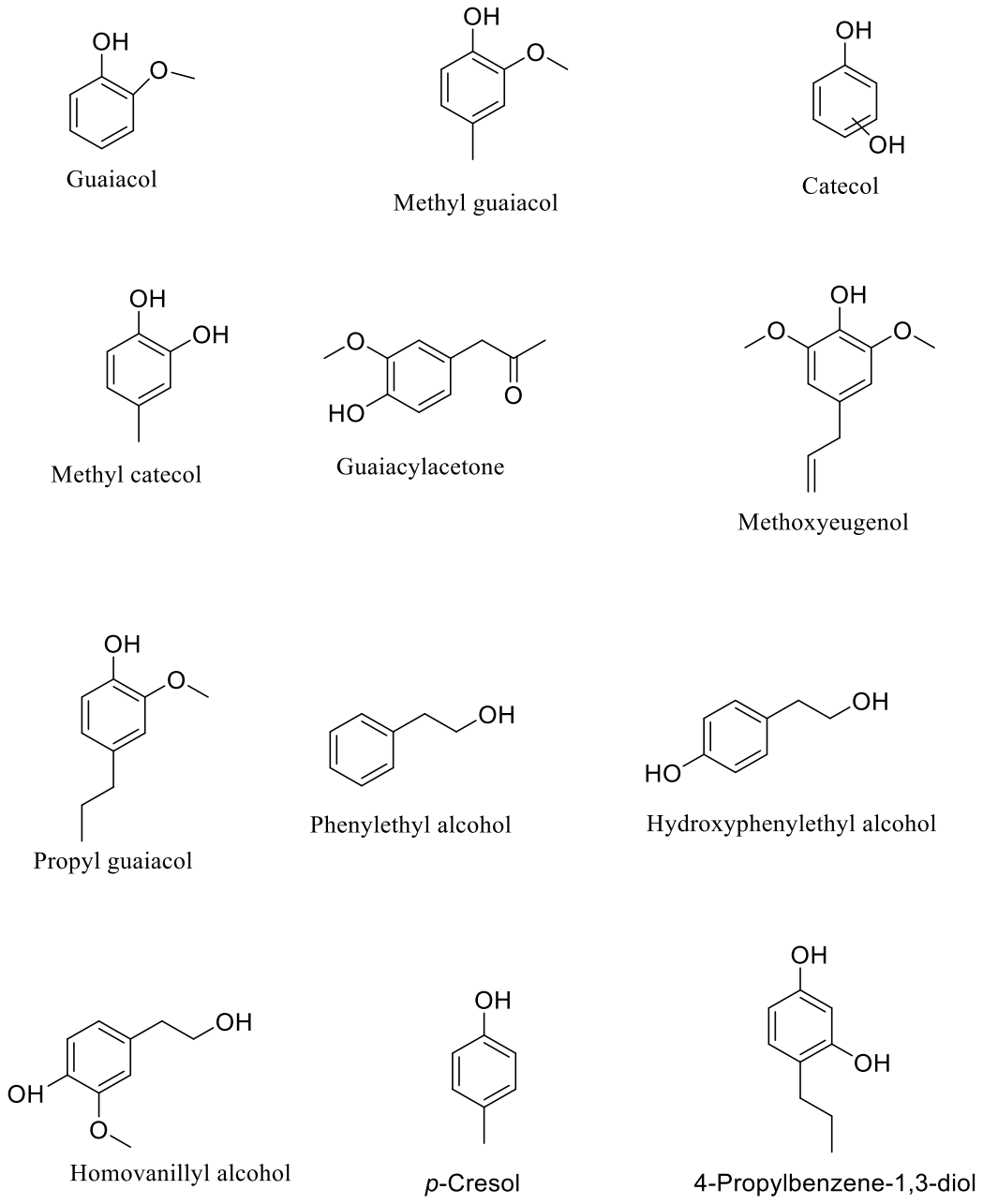


Figure 2.8: Selected chemical compounds found in lignin derived bio-oils.

The structures were retrieved from http://www.sigmaaldrich.com/technical-service-home/product-catalog.html and redrawn with ChemDraw.

3.0 CHARACTERISATION OF COMPLEX MIXTURES

3.1 Liquid chromatography and complex mixtures

Chromatography is a physical method for separation of mixtures which uses the principle of the differences in surface interaction between analytes and eluent molecules to separate compounds within one sample. Liquid chromatography (LC) is therefore a separation technique in which the mobile phase is a liquid.

There are basically two major types of LC system, the reverse and normal phase chromatography. In reversed-phase (RP) the mobile phase is significantly more polar than the stationary phase. It is therefore named reverse phase because in normal-phase liquid chromatography, which was developed first, the mobile phase is significantly less polar than the stationary phase. In RP-LC, hydrophobic molecules in the mobile phase tend to adsorb to the relatively hydrophobic stationary phase. Hydrophilic molecules in the mobile phase will tend to elute first. Separation columns typically comprise a C8 or C18 carbon-chain bonded to a silica particle substrate. The normal-phase chromatography consists of a more polar stationary phase with a non-polar mobile phase. Typical stationary phases for normal-phase chromatography are silica and some bonded normal phase material with organic moieties such as cyano and amino functional groups [23].

In a high-performance liquid chromatography (HPLC) system, the analytical system is composed of two phases. The stationary phase (absorbent) is the solid support situated within the column often packed with silica bonded hydrocarbons. The mobile phase (eluent) refers to the solvent which is continually added to the column. When injecting a sample into the column, it will migrate according to its affinity to the mobile and stationary phases, this means compounds that have less strong affinity to the stationary phase will elute faster than compounds that have stronger affinity to the stationary phase [23, 24]. In reverse phase chromatography, which is the most common type of chromatography, the elution order is from polar to weakly polar and the non-polar compounds elutes last from the column. In a HPLC system, the eluent is continuously pumped through the column at a high pressure, which ensures that the analytes and the mobile phase are forced through the densely packed column.

Furthermore, liquid chromatography is often coupled to a mass spectrometry (MS) instrument to obtain a hyphenated set up, liquid chromatography mass spectrometry (LC-MS otherwise called HPLC-MS). This form of hyphenated analytical technique combines the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry (MS). See Figure 3.1 for a simplified typical hyphenation of LC with a MS system.

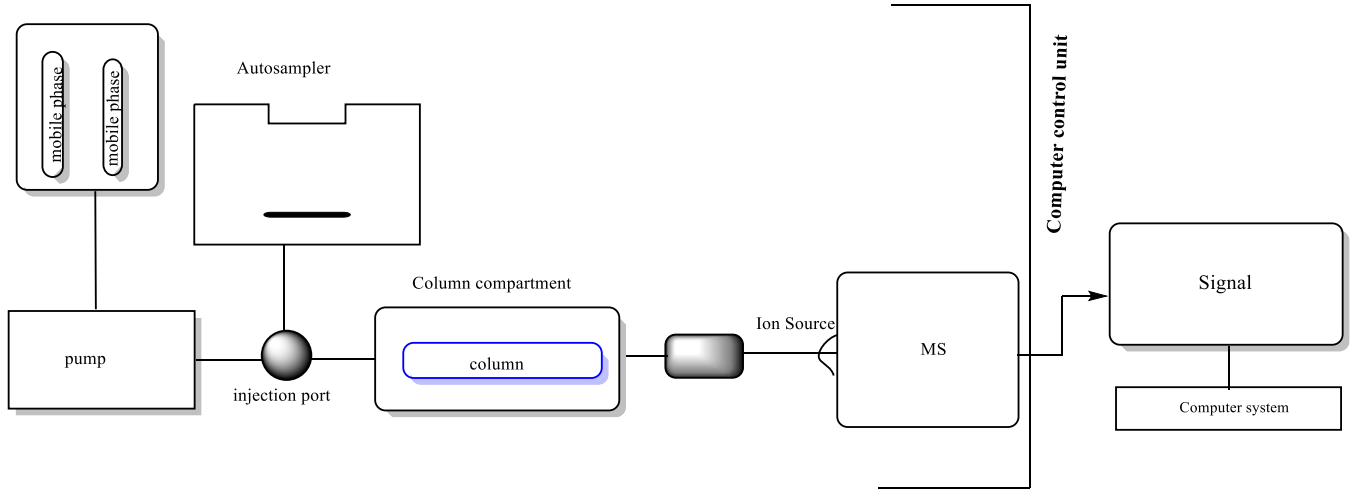


Figure 3.1: Typical set up of LC system connected to a mass spectrometry instrument [23].

Chromatography is the foremost technique applied in separation and analyses, and thus it is the ultimate proven method for separation and characterisation of complex mixtures into their constituent parts and it is therefore the most important procedure for isolating and characterisation of complex mixtures such as petroleum and bio oil mixtures. Consequently, chromatographic techniques have been employed over time in the study and analysis of petroleum and renewable bio-oils [8, 10, 25-29].

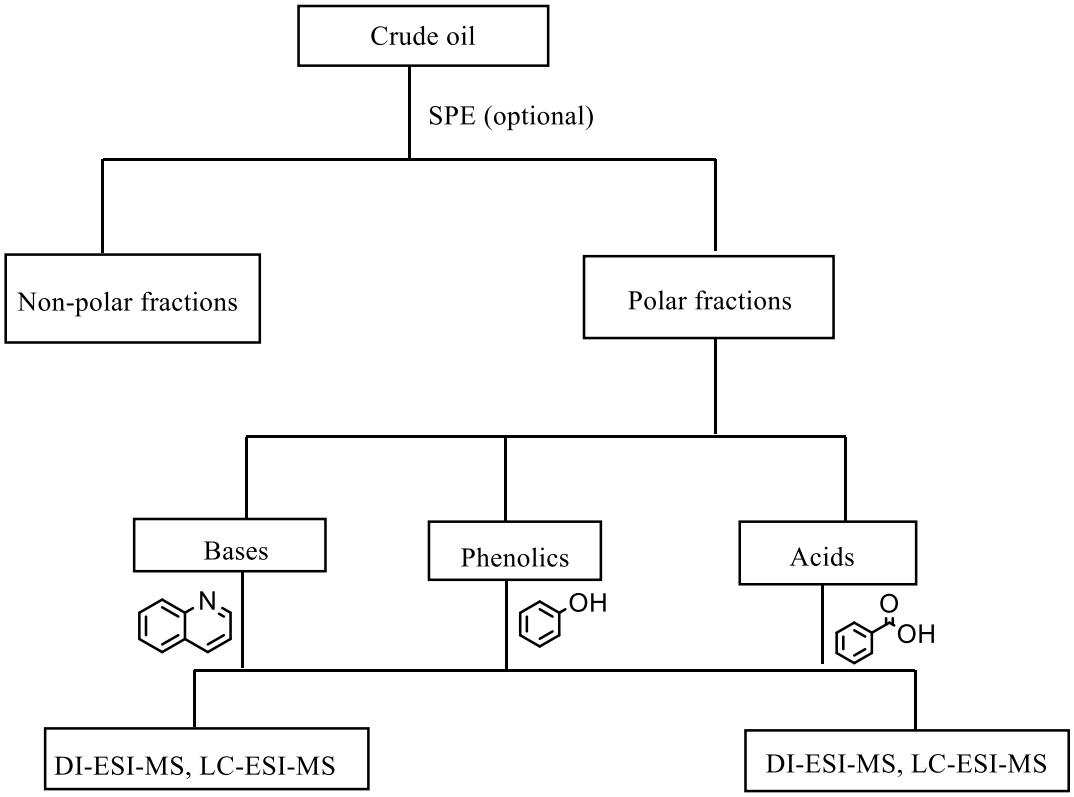


Figure 3.2: Flow of fractionation in characterisations of crude oils.

Biofuels contain acidic compound comparable to those found in crude oils and thus such compound's behaviours are analogous to petroleum acids and its components.

Figure 3.2 shows a typical process flow of characterisation of complex mixtures such as petroleum and biofuels using liquid chromatography and/mass spectrometry.

3.2 Mass Spectrometry (MS)

Mass spectrometry (MS) is an analytical technique that produces spectra and measures the mass-to-charge ratio (*m/z*) of the atoms or molecules of a sample. The spectra are employed to determine the elemental or isotopic identity and composition of a sample, the masses of particles and of molecules, and to elucidate the chemical structures of molecules in wide variety of complex mixtures. Typically, a mass spectrometer is a stand-alone instrument and most often connected to a chromatograph and a computer as previously highlighted in section (3.1), see Figure 3.2. The chromatograph separates the mixtures and introduces the sample into the mass spectrometer. A MS works by ionizing the analytes molecule to generate charged molecules or molecule fragments, then separates and detects the resulting ions by measuring their mass-to-charge ratios (*m/z*). The computer system controls the operation of the chromatograph and the MS system, and provides data manipulation and storage during and after data collection.

Contemporary and sophisticated analytical techniques such as mass spectrometry generate plethora of data that suggests the composition and properties of crude oils and crude oil fractions [30]. In mass spectrometry, analyses can be done in positive or negative polarity depending on the chemistry of the target compounds. In positive ion mode $[M+H]^+$ /[M+ Adducts]$^+$ the analyte is sprayed at low pH to encourage positive ion formation. In this case, the number of charged species typically observed in an electrospray spectrum is reflected in the number of basic sites on a molecule that can be protonated at low pH. In negative ion mode $[M-H]^-$ the analysis is normally carried out well above the molecule's isoelectric point to deprotonate the molecule [31, 32].

A variety of ionisation techniques are used for mass spectrometry. The most important consideration is the internal energy transfer during the ionisation process and the physico-chemical properties of the analytes that can be ionised. Some ionisation techniques are very energetic and causes extensive fragmentation, while others are softer and only produce ions of the molecular specie; a typical example of such soft technique is the electrospray ionisation (ESI) which is discussed in more detail later. Electron ionisation, chemical ionisation and field ionisation are only suitable for gas-phase ionisation and thus their use is applied in mainly volatile and thermally stable compounds. These direct ion sources exist under as two types: liquid-phase ion sources and the solid-state ion sources [31, 32]. Figure 3.3 outlines a typical set up of a MS instrument.

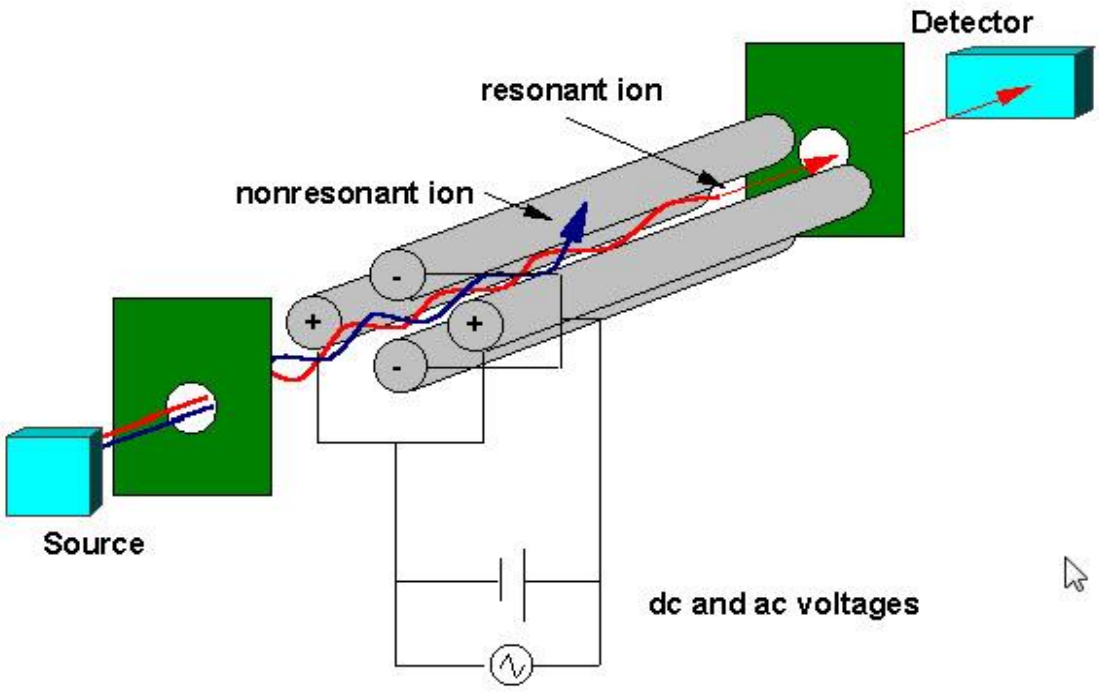


Figure 3.3: A typical diagram of a quadrupole mass analyser (centre) in a MS-system. The ion travels from the source, through the 4 metal rods arrangement in a unique oscillating pattern, and reaches the detector. *Retrieved February 3 2017, from http://www.cif.iastate.edu/mass-spec/ms-tutorial.*

In the solid-state ion sources, the analytes are in a non-volatile deposit. It is obtained by various preparation methods which frequently involve the introduction of a matrix that can either be a solid or vicious fluid. This deposit is then irradiated by energetic particles or photons that desorb ions near the surface of the deposit. These ions can be mobilised by the electric field and focused towards the analyser. Matrix-assisted laser desorption, secondary ion mass spectrometry, plasma desorption and field desorption sources uses this type.

In liquid-phase ion sources such as the ESI, the analytes are in solution [33]. The solution is introduced by nebulisation, as droplets into the source where ions are produced at atmospheric pressure and focused into the mass spectrometer through a vacuum pumping stage. Electrospray, atmospheric pressure chemical ionisation and the atmospheric pressure photoionization sources are all typical examples of this type. Principally, most ion sources produce ions mainly by ionizing a neutral molecule (Figure 3.4) in the gas phase (ESI in liquid

state) through electron ejection, electron capture, protonation, deprotonation, adduct formation or by the transfer of a charged species from a condensed phase to the gas phase.

As mentioned earlier there are several types of ion sources used in mass spectrometry, but in this work, we will focus on the electrospray ionisation as the ion source of choice for our purpose. The reader may consult [31, 32] for more details on the other types of ion sources and their working principles.

Primarily electrospray ionisation (ESI) works by converting the HPLC effluent, already containing the sample, into an aerosol subjecting the resulting spray into high voltage chamber held under atmospheric pressure as can be seen in Figure 3.4. This process creates a mist of charged droplets that flow towards the tip opening of the capillary. The electric field is obtained by applying a potential difference of 1-5 kV between the capillary and the counter-electrode, separated by 0.3-2 cm, producing order of 106 V m^{-1}. This field induces a charge accumulation at the liquid surface located at the end of the capillary which will break to form highly charged droplets [31, 32].

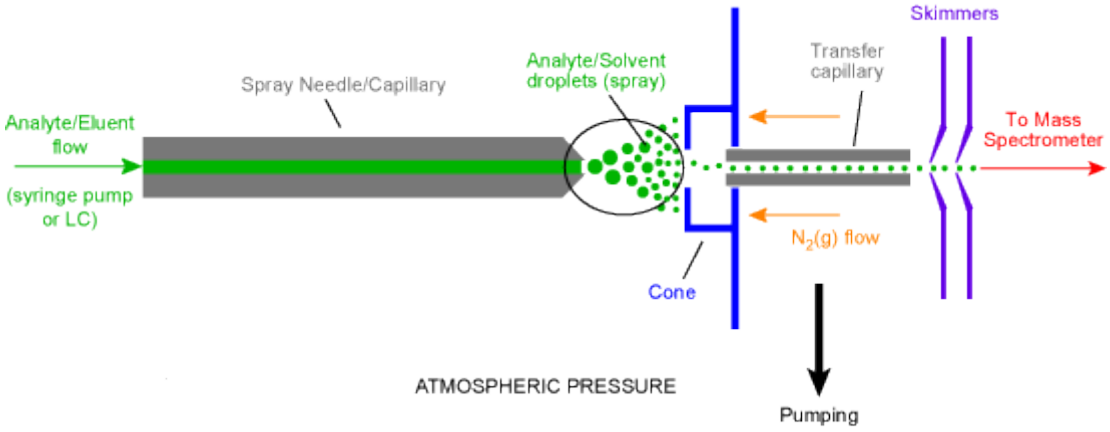


Figure 3.4: A typical schematic of an electrospray ionisation interface. As the charged droplets travel towards the capillary opening, they are subjected to the counter flow of a drying gas, such as nitrogen (N_2), which causes evaporation of solvent molecules from the droplets. *The scheme was retrieved January 25, 2017, from http://www.bris.ac.uk/nerclsmsf/techniques/hplcms.html*.

By way of the charged droplets travel towards the capillary opening, they are subjected to the counter flows of a drying gas, usually nitrogen (N_2) or through a heated capillary (Figure 3.4) to remove the last solvent molecules and this causes evaporation of solvent molecules from the droplets.

Evaporation continues until electrostatic repulsions between the increasing concentrated charges cause the droplets to break apart. The three processes; evaporation, charge concentration, and droplet disintegration continue till the analytes ions are finally desorbed into the vapour phase, passed into the sampling capillary, then into the high vacuum of the MS analyser [31, 32]. The ionisation in ESI can be tuned to either negative, $[M\text{-}H]^- + H^+$ or positive, $M + H^+ \rightarrow MH^+$ polarity. ESI allows very high sensitivity to be reached and it is easy to couple to HPLC for separation and identification of compounds in complex mixtures such as petroleum and bio oils.

3.3 ESI-MS application in complex mixtures

Mass Spectrometry (MS) has been applied over the years in the analysis of organic compounds including petroleum. It is one of the foremost analytical techniques associated with the identification of chemical components in complex mixtures such as petroleum [15, 25-27, 29].

Hyphenated analytical techniques, for instance, mass spectrometry (MS) coupled to chromatographic techniques such as gas chromatography (GC-MS), liquid chromatography (LC-MS), Fourier Transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) [27] have been employed in the study and analysis of petroleum samples. By choosing between different ionization methods such as ESI, APCI etc. and using different mass analyser (time-of-flight, TOF, ion-trap and tripled quadrupole mass analysers as used in this study, it is possible to obtain valuable information to identify the analytes under study. By coupling MS and LC (LC-MS), signals are obtained with characteristic retention times which in most cases would be related to the molecular mass (*m/z*) of the analytes.

Furthermore, LC-MS allows quantification, and it is a reproducible technique which produces characteristics fragmentation patterns of the analytes thus enhancing their identification. Electrospray Ionisation (ESI) for example is a well-known and common technique for ionisation of polar organic molecules [34-36] such as organic acids. The ionisation can be in either negative mode [M-H] or in positive mode [M+H]. In each case a proton is gained or lost from the parent compound respectively.

Nevertheless, petroleum and its refined products are mostly analysed by GC-MS, thus LC-ESI-MS is an evolving field in the study of the petroleum and its components [37]. Electrospray ionisation mostly targets polar compounds in the complex petroleum mixture, most of these compounds contains heteroatoms and often poses environmental concern. These group may include but not limited to basic and neutral nitrogen compounds, amides, naphthenic acids, and phenols in petroleum as well as in biofuels. The specific targeting helps to simplify the mass spectrum by eliminating other hydrocarbon matrix of less interest [27].

In this present work, the resolution and accuracy of the developed method needs to directly resolve and designate mass to charge ratios of interest in the crude petroleum and bio-oil materials. Furthermore, through the direct ESI-MS injection (DI-ESI-MS), several hetero aromatic compounds have been identified both in positive (basic species) and negative (acidic

species) modes analysis of crude oil samples. Chemical characterisation of complex mixtures such as petroleum often poses onerous challenge. The enormous challenge lies in developing suitable, rapid, reliable and objective methods for the analysis of such complex mixtures [38]. The present work outlines the development of liquid chromatography electrospray ionisation mass spectrometry based analytical method for assessing the identity of petroleum acids in normal and biodegraded petroleum hydrocarbon mixtures as well as in lignin derived biofuels.

3.4 Solid Phase Extraction

Sample preparation or pre-treatment in analytical chemistry denotes the various ways in which a sample is treated prior to its analysis. Preparation of sample is a vital step in most analytical techniques, since the techniques are most frequently not responsive to the analyte in its in-situ form, or the results are distorted by interfering species. Getting the right and the most suitable extraction procedures prior to analyses is often a challenge to analytical chemists. The analytical chemist wants to get the extraction procedure suitable for the type of analytical instrument, and which is compatible with the type of chromatography to be run. Owning to that, sample preparation suitable for a reverse phase chromatography may not be suitable for a normal phase chromatography or perhaps the target molecules of interests may differ.

There are many ways to prepare samples for onward application to LC-ESI-MS analyses. The most common form of extraction is the liquid-liquid extraction (LLE) and the solid phase extraction (SPE) procedures. SPE is a sample preparation process by which compounds that are dissolved or suspended in a liquid mixture are separated from other compounds in the mixture according to their physical and chemical properties [23]. The Process is similar, to that which occurs on the stationary phase of analytical column during LC run.

The solid phase extraction, (Figure 3.5) is used to clean up samples by sorbing impurities on a solid phase contained in a column or tube while the analyte is eluted from the tube.

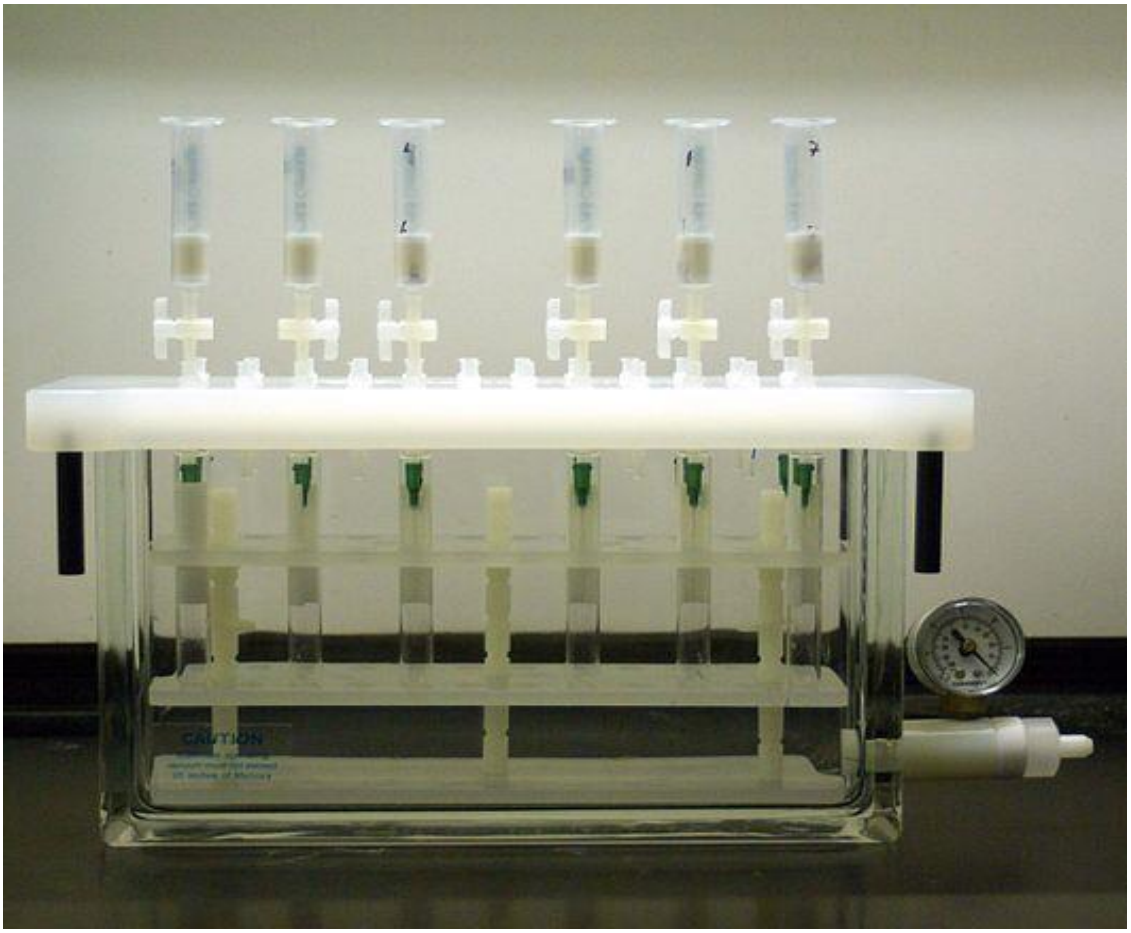


Figure 3.5: A typical solid phase extraction set-up. The solvents from the cartridges drip into the chamber below, where tubes collect the effluent. A vacuum port with gauge is used to control the vacuum applied to the chamber. *Retrieved February 20 2017, from https://en.wikipedia.org/wiki/Solid _phase_extraction.*

Conversely, the analytes can be sorbed on the solid, allowing the rest of the sample to pass out of the tube. Whichever way, the impurities are separated from the analytes of interests. The sample can be a liquid or solid dissolved in a solution. The process is like liquid chromatography which has been discussed earlier in section (3.1), especially the affinity chromatography, and many solid stationary phases (silica, cyano etc.) in HPLC find use in SPE. SPE has many advantages why it has become method of choice, such advantages include improved throughput, higher and more reproducible recoveries, cleaner extracts and no likelihood of formation of emulsions as maybe the case in LLE. In a typical solid phase extraction, as in Figure 3.5, the cartridges drip into the chamber, where tubes collect the effluent. A vacuum port with gauge is used to control the vacuum applied to the chamber.

3.5 Data Analysis

Mass spectrometry data is commonly represented in form of a chromatogram showing each separated compound on a retention time axis and a mass spectrum for each compound. The chromatogram includes the total ion current (TIC), the selected ion monitoring (SIM), and the selected reaction monitoring (SRM). Other types of mass spectrometry data can be represented as a three-dimensional contour map. In this arrangement, the mass-to-charge ratio, *m/z* is on the x-axis, intensity on the y-axis, and an additional experimental parameter, such as time, is recorded on the z-axis.

Furthermore, in MS data analysis is specific to the type of experiment producing the data. Thus, understanding the nature of the target analytes are therefore fundamental to identifying the corresponding spectra. As discussed previously, many mass spectrometers work in either negative ion mode or positive ion mode. It is very central to know whether the observed ions are negatively or positively charged. This is often important in determining the neutral mass but it also indicates something about the nature of the molecules.

Different types of ion sources result in different types of fragments produced from the original molecules. An electron ionization source produces extensive fragmentation, commonly single-charged radicals, whereas an electrospray source as used in this study, typically produces non-radical quasimolecular ions that are multiply or singly charged. Tandem mass spectrometry purposely produces fragment ions post-source and can considerably change the sort of data achieved by an experiment. [31, 32]. In a low-resolution mass spectrometry, a mass-to-charge ratio value (*m/z*) with only integer precision can represent an enormous number of theoretically possible ion structures but in a high resolution which may have up to four decimal places such compounds can be specifically identified.

4.0 METHODS AND EXPERIMENTAL DESCRIPTION

4.1 LC-MS Instrument

The instrument is an Agilent 6420A triple quadrupole (QqQ configuration) mass analyser using electrospray ionisation (ESI) interface. It is connected to an Agilent 1200 series LC module (binary pump, column compartment/oven and auto sampler). The instrument is set up in way that the samples can be introduced to the spray chamber either through the LC column or by flow injection (no LC column).

4.2 LC Column

4.2.1 ZORBAX Stable Bond (SB)

The LC column primarily installed on the instrument is an Agilent ZORBAX SB-C18, RRT; 2.1× 50 mm, 1.8 µm. ZORBAX SB-Aq is an LC alkyl reversed-phase bonded phase designed to retain hydrophilic and other compounds when using highly aqueous mobile phases as in the present work. The ZORBAX columns is made using silanes that sterically protect the siloxane bond. According to the information from Agilent [39], acid labile endcapping reagents are not used. The result is vastly improved column life and extraordinary chemical and temperature stability. There are different lengths and particle size for this type of column, but in this work a C-18 with 1.8 µm internal diameter has been used. According to information from Agilent, excellent peak shape is expected for many basic compounds. The surface chemistry is designed to allow the alkyl phase to remain fully accessible in highly aqueous mobile phases, preventing "phase collapse.” Because of this, separations on SB-Aq are reproducible from run to run over long column lifetimes [39].

4.2.2 Poroshell 120

The second column used is a Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm LC column from Agilent Technologies, USA. The Poroshell 120 columns is made to provide exceptional efficiency and reliability, significantly boosting performance, when separation efficiency and reprioducibilty is the goal [39]. There are mainly three different partcile sizes for this type of column but in this work a 2.7 µm internal diameter has been used and this particle size was sufficient to provide the separation needs.

4.3 SPE column

The SPE column is Isolute SPE cyano (end-capped), international sorbent Technology, UK, with a 1000 mg sorbent mass, and 6mL reservoir volume.

4.4 Equipment

Syringes (0.5ml, 1ml, 2ml and 3ml), weighing balance, Eppendorf micro pipette, glass pipettes, glass sample holder, spatula, measuring cylinders (50 ml, 100 ml, 500 ml and 1L), HPLC vials, 20ml sample bottles, fume hood, and water bath.

4.5 Solvents

HPLC-grade acetonitrile (ACN), dichloromethane (DCM), formic acid, acetic acid, hexane, and methanol (≥ 98 purity) were from Fluka Chemie (Basel, Switzerland). De-ionised water was purified in a Milli-Q system (Millipore, Milford, MA, USA).

4.6 Reagent standards

Guaiacol glyceryl ether standard ≥ 98% purity, dihydroxybenzene > 99% purity, dihydroxybenzoic acid ≥ 99% purity, benzoic acid ≥ 99.5% purity, naphthol ≥ 99% purity, methyl catecol ≥ 98% purity, ethyl catecol ≥ 95% purity, amino benzamide ≥ 98 purity, were purchased from Sigma-Aldrich Co. (St. Louis MO USA). naphthoic acid > 97% purity, methyl benzoic acid > 99% purity, quinolinol > 97% purity, carbazole ≥ 98% purity, amino phenol > 98% purity, amino benzoic acid ≥ 98% purity were purchased from Fluka Chemie (Basel, Switzerland).

4.7 Standards preparation

Stock solutions (ACN as solvent) up to 10mg/mL were made from each of the standard samples from section 4.6, also see Table 4.1. The stock solutions were diluted to appropriate concentrations, 20 µg/ml, 50 µg/ml, 100 µg/ml, and 200 µg/ml per needs. See Table 4.1 for the list of standards showing their molecular weights and possible ionisation. Figure 4.1 shows the molecular structures of the standards. The background for choosing the list of standard compounds (Table 4.1) is on the assumption that the oils contain same and/or analogous compounds.

Table 4.1: Standards showing molecular weights and their likely ionisation modes.

S/N	Standard compounds	Molecular weights (g/mol)	Polarity	*m/z*
1	Naphthoic acid	172.18	-	171
2	Naphthol	144.17	-	143
3	Methyl Benzoic acid	136.16	-	135
4	Guaiacol	124.14	-	123
5	Guaiacol glyceryl ether	198.22	+,-	199, 123
6	NH_2 Phenol	109.13	+, -	110, 108
7	Catecol	110.10	+, -	111, 109
8	NH_2-Benzamide	136.15	+	137
9	Quinolinol	145.16	+, -	146, 144
10	Carbazole	167.20	+,-	168, 166
11	Benzoic acid	122.12	-	121
12	Dihydroxybenzene	110.10	-	111, 109
13	Dihydroxybenzoic acid	154.12	-	153
14	Methyl catecol	124.13	-	123
15	Ethyl catecol	138.16	-	137

***positive and negative ionisation is designated (+) and (-) respectively.*

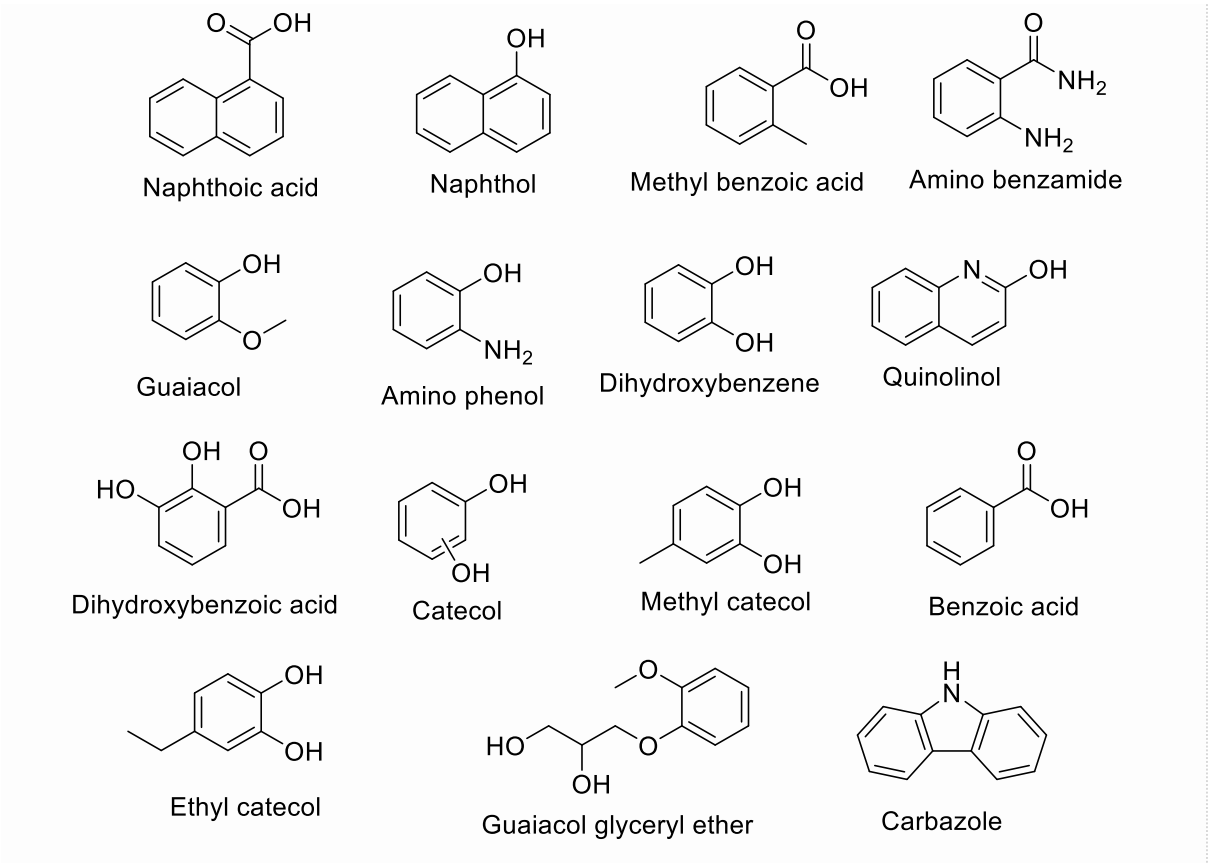


Figure 4.1: Molecular structures of standards in Table 4.1

4.8 Petroleum and Lignin bio-oil samples

The petroleum samples originated from Norwegian continental shelf and supplied by the Norsk Hydro ASA and Statoil ASA. The oils are marked with names per their production fields, e.g. *Heidrun, Snorre* and *Grane*.

Aliquots of the three petroleum samples were removed from the stock after heating to 60°C for 60 mins in a water bath, the samples were homogenised by shaking to ensure true representative composition of each sample. About 5mL each were taken from each sample into a 20mL sample bottle and labelled accordingly. The bulk oils were returned to the refrigerator and the collected quantities were also kept in the refrigerator until when needed for further preparations.

The bio-oil samples are gotten from the ongoing research in our group where different thermochemical approaches are employed in converting lignin fractions of the fuel to bio-oils, using different depolymerisation techniques such as water or ethanol as reaction medium and formic acid as hydrogen donor, the details of the bio-oil origin and their reaction conditions are detailed in Table 4.2. The conversion procedure was performed by thermochemical and solvolytic processes, detailed description of the process and process parameters, including feedstocks is described elsewhere [21]. The conversion was performed by Hilde Halleraker and Camila Løhre.

Table 4.2: Details of bio-oil samples and their reaction conditions.

Oil code	**Type of lignin**	**Temperature (°C)**	**Reaction media**	**Reaction time (hr)**	**Pre-treatments**
L.1.3A	Softwood	320	Water/formic acid	2	Kraft process
L.1.4A		360	Water/formic acid	2	
L.4.3A	Hardwood	320	Water/formic acid	2	Milox process
L.4.4A		360	Water/formic acid	2	
AS.II.3.2	Birch	360	Water/formic acid	2	Enzymatic hydrolysis, tempered at 210°C for 10mins
AS.II.E.3.2		360	Ethanol/formic acid	2	

4.9 SPE procedure

In the sample preparation procedure, the SPE column (4.0) is wetted with the first eluent, approximately 30mg of samples (4.8) were taken from the sample bottle, dissolved in 0.075mL of DCM:MeOH 93:7 (v/v) and applied to the SPE column. The solvents used are as follows: hexane:DCM 90:10 (v/v), hexane:DCM 90:10 (v/v), ACN 100% and MeOH 100%. The procedure was adapted and modified from [10]. The details of the solvent composition and the approximate volume of each are detailed in Table 4.3 below.

Table 4.3: Fractionation procedure for the SPE extraction: Fractionation procedure for the SPE extraction.

Fractions	**Solvent compositions**	**Approximate volume (mL)**	**Compound types**
1	Hexane:DCM 90:10 (v/v),	20	Non-polar fractions
2	Hexane:DCM 90:10 (v/v),	10	Non-polar fractions
3	ACN 100%,	15	Intermediate polar fractions
4	MeOH 100%.	15	Highly polar fractions

Figure 4.2: SPE step during the fractionation petroleum samples.

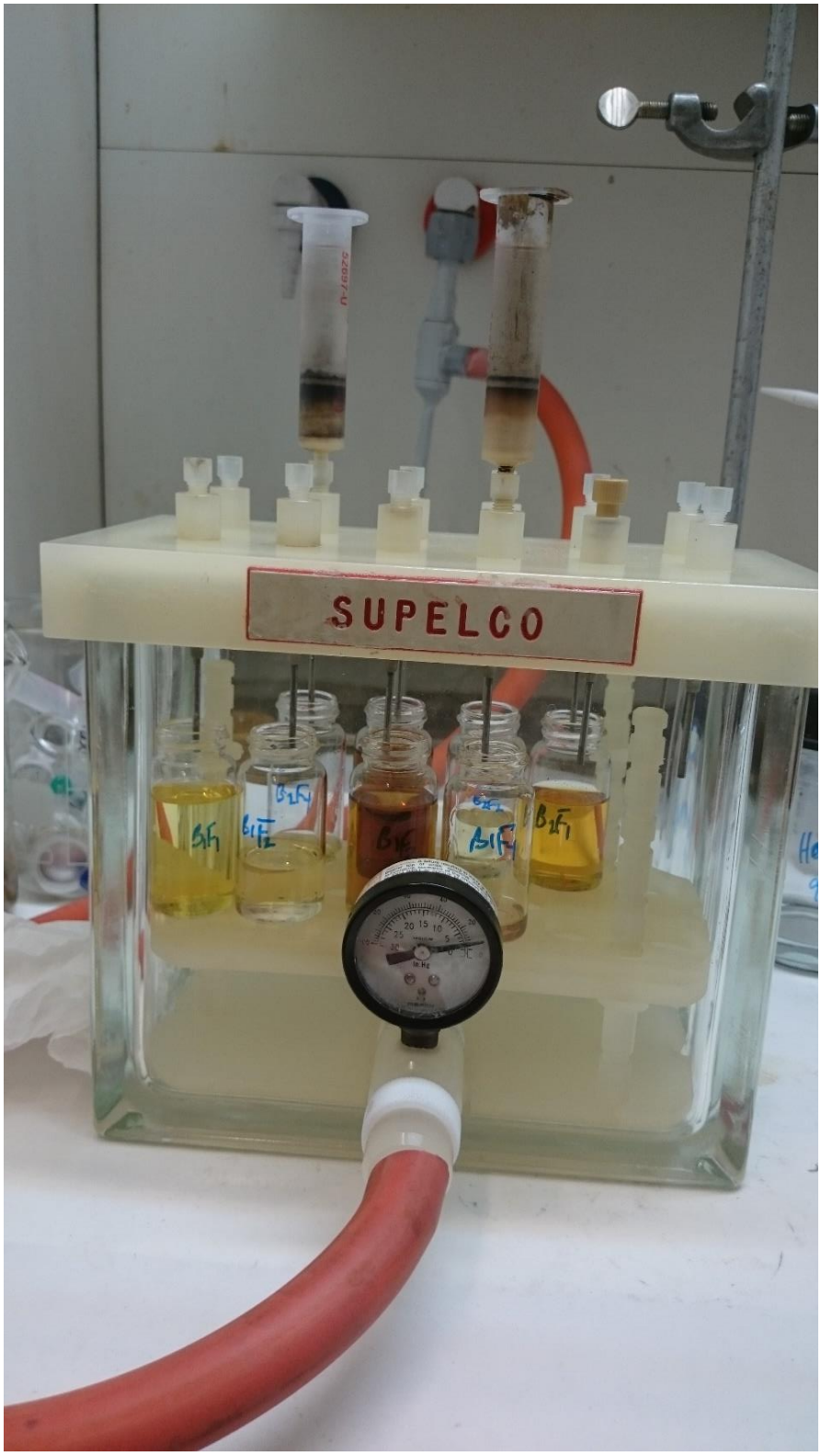


Figure 4.3: SPE step during the fractionation of bio-oil samples.

After each solid phase extraction procedure as demonstrated in Figure 4.2-4.3, the solvents in the various fractions were evaporated under nitrogen gas flow (N_2-gas) until they were completely dried. The bottles were reweighed and documented to check for recoveries. The relevant fractions were reconstituted in acetonitrile to the relevant concentration before DI-ESI-MS and/or LC-ESI-MS analyses. Petroleum and bio-oils are complex mixtures (2.0) containing several hundreds of various types of compounds and this work is mostly focused on the mostly poplar fractions. Fractions 1 and 2 were performed to wash off the non-polar fractions in the samples, in the case of crude oils, to wash off the hydrocarbon components of the crudes and other non-polar components. Figure 4.4 outlines a concise process scheme of the fractionation procedure.

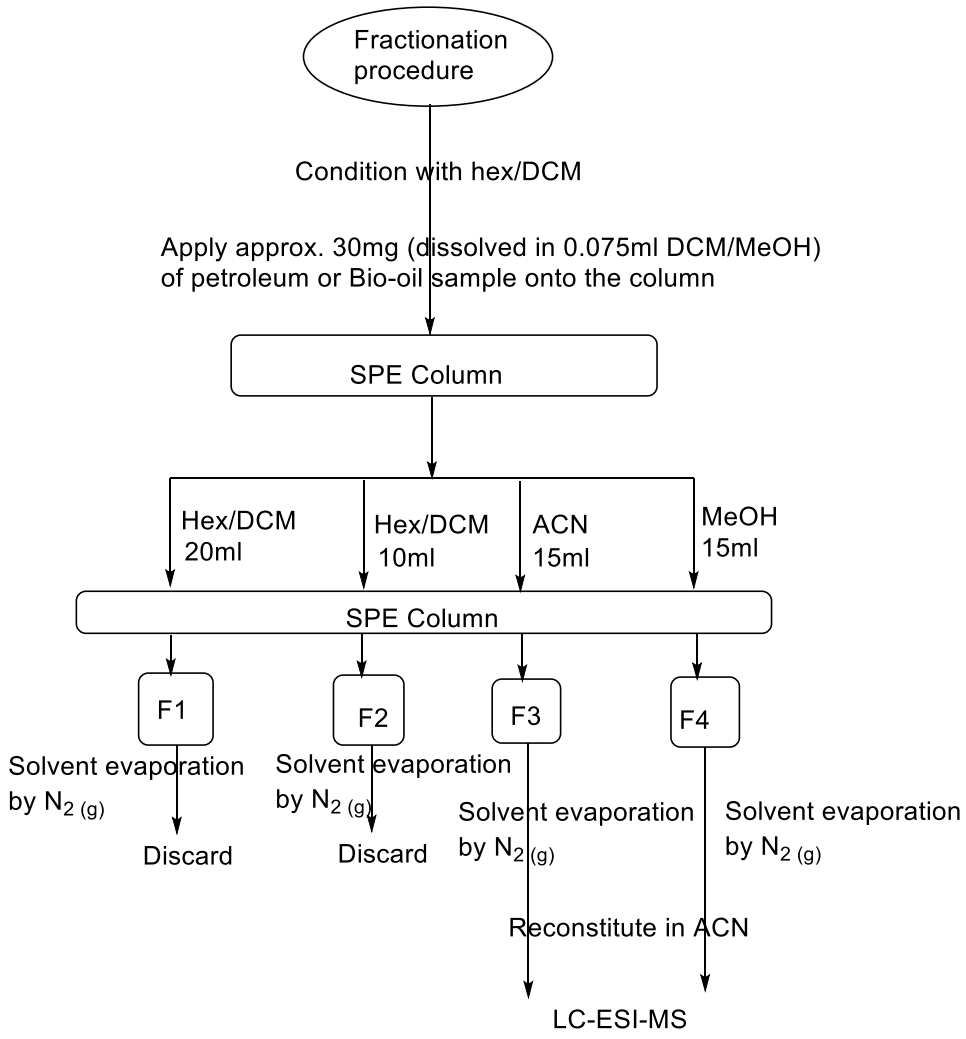


Figure 4.4: Flow scheme of fractionation procedure.

4.10 Fingerprinting procedure

The fingerprinting approach were performed by dissolving the aliquots of the different oils (4.8) in acetonitrile. The concentration of the whole samples injected into the MS instrument was 200µg/ml and the injection volume was 2µL. The samples were submitted to the LC-ESI-MS and analysed in both positive and negative mode polarity. See Figure 4.5 for the process scheme.

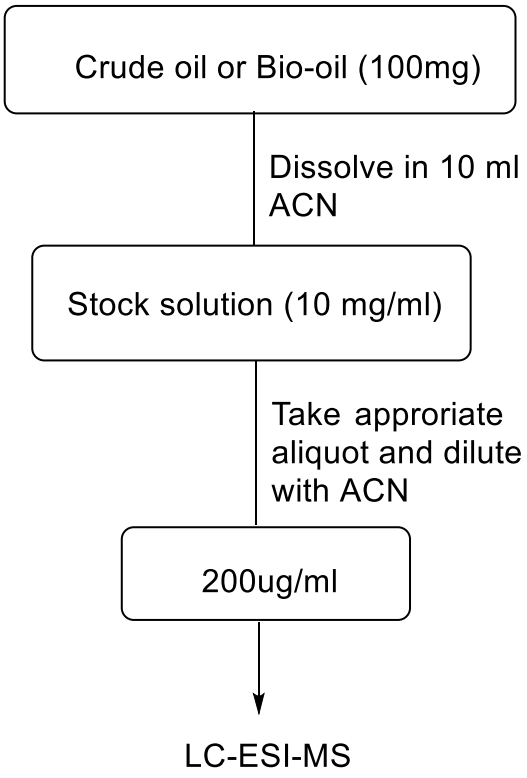


Figure 4.5: Flow scheme of the fingerprinting procedure.

5.0 RESULTS

5.1 LC-MS method development

Commercial standard compounds (Table 4.1) were used to develop the liquid chromatography mass spectrometry method.

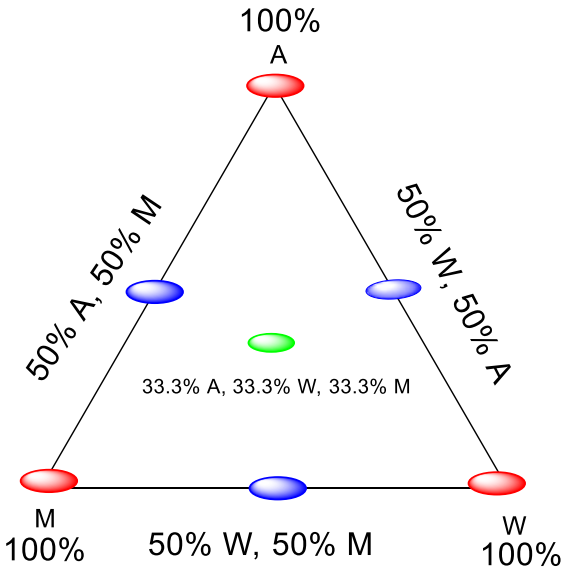
5.1.1 Preparing the LC

Prior to every run and sequence, the column is equilibrated between 45 to 60 mins.
100% of [$A_{2,}$ H_2O] is run from 15-20mins and 100% of [$B_{1,}$ ACN] is run from 15-20mins.
The starting gradient program, 85% of [$A_{2,}$ H_2O] and 15% of [$B_{1,}$ ACN, 0.1% acetic acid] is run from 15-20min. This is done to load the column with the right composition of the gradient program to enhance both retention of the compounds and the separation with minimal co-elution and peak overlap if any.

5.1.2 Solvents selection

Based on literature on the separation of the target analytes in the mixture, three candidates for mobile phase solvents were identified: water, methanol and acetonitrile.These solvents are most commonly used for a reverse phase liquid chromatography.

In this study, experimental design using solvent triangle Figure 5.1 was applied to find the best mobile phase composition and/or the region were separation is more likely to occur. The three mobile phases designated as 100% W, 100% M, and 100% A in the solvent triangle [Figure 5.1], along with three 50:50 binary mobile phases and a centre point of 1/3:1/3:1/3 ternary mobile phase make up the seven experiments.



M= methanol, A= acetonitrile, W= water

Figure 5.1: Solvent mixture triangle, showing different experimental points. This was done in column 1, see Table 5.3. Every other optimisation process was run this column unless otherwsie stated.

The starting point was chosen by adjusting the compositions of methanol/acetonitrile, and methanol/water. The data in Figure 5.1 was used to estimate the composition of methanol/acetonitrile and methanol/water mobile phases that would produce possible separation in similar analysis times and appreciable retention times.

The next point was chosen by adjusting the amount of acetonitrile and water in a ACN/H_2O mobile phase, producing the best possible separation within the desired analysis time.

By evaluating the chromatograms of the standards from the mobile phases, it was found out that a region of possible separation occurs within water and acetonitrile compositions. Some of the chromatograms from the solvent composition are shown below. See Figure 5.2 shows the chromatogram of methanol: water: 50/50 (v/v), Figure 5.3 shows the chromatogram of methanol:acetonitrile 50/50 (v/v), Figure 5.4 shows the chromatogram of water:acetonitrile: 50/50 (v/v). These regions were further optimised to get the adequate composition for the gradient program.

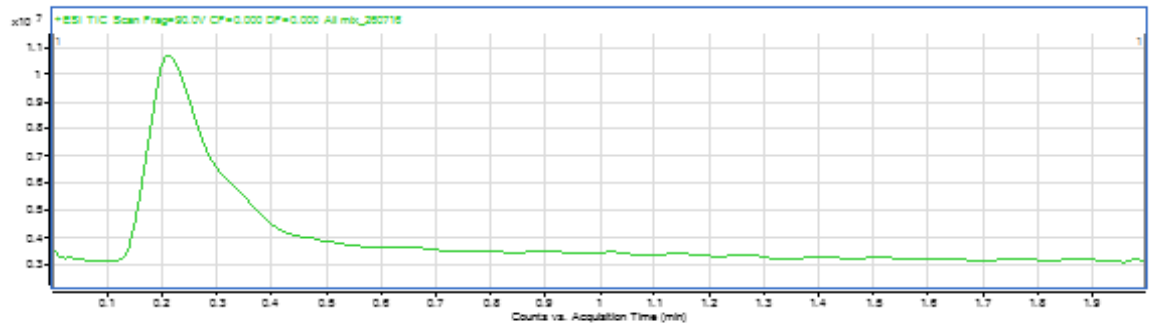


Figure 5.2: TIC of gradient program using methanol: water: 50/50 (v/v). The mixture contained most of the compounds in Table 4.1.

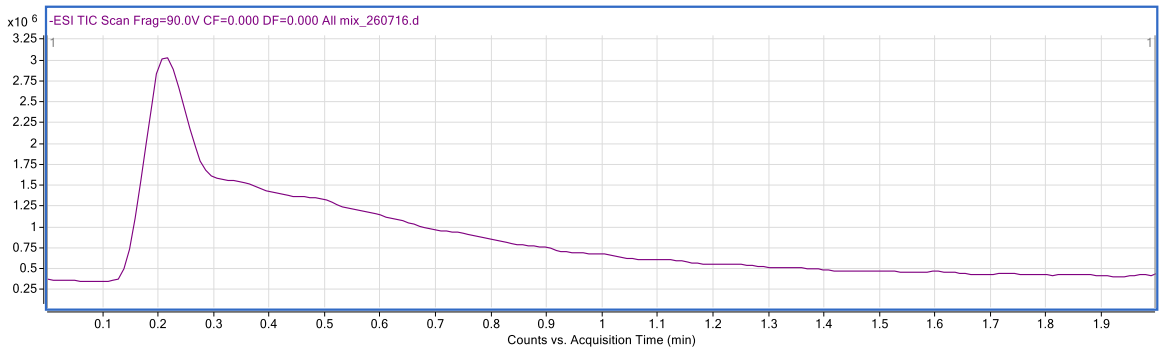


Figure 5.3: TIC of gradient program using methanol: acetonitrile: 50/50 (v/v). The mixture contained most of the compounds in Table 4.1.

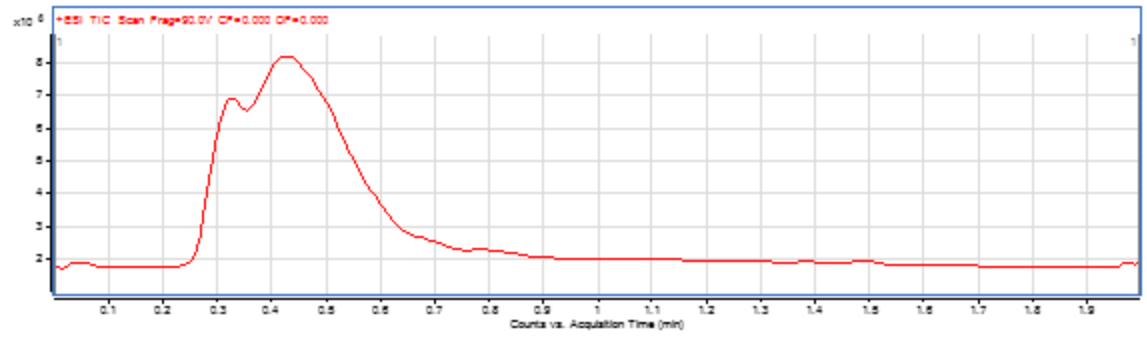


Figure 5.4: TIC of gradient program using water: acetonitrile: 50/50 (v/v). The mixture contained most of the compounds in Table 4.1.

5.1.3 Retention of analytes

The retention of polar compounds containing amine hydrophilic groups, such as -NH_2, -$COONH_2$ groups, on the column are weak. These group of polar compounds generally show poor retention in the column having between 0.5 to 3 mins (Figure 5.5) when compared to the compounds which contain such groups as: -COOH,-OCH_3. The retention times for these group of polar compounds ranges from 2.8mins to 5.8 mins, see Figure 5.6. Table 5.1, summarises the functional groups and their range of retention in the column.

Table 5.1: shows list of compound function groups and their range of retention times in the column.

Functional groups	**Retention times (mins)**
-NH_2, -$COONH_2$	0.5-3
-COOH, -OH, -OCH_3	2.8-5.8

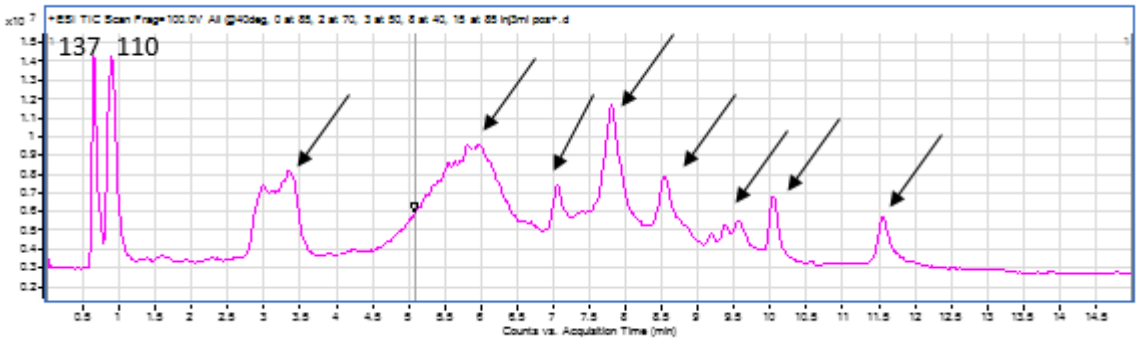


Figure 5.5: TIC to illustrate poor retention of compounds with basic functional groups. Peak *m/z* 137 represents amino benzamide and 110 = amino phenol all eluting in less than 1 min. The peaks on black arrows are unknown peaks. The ESI was operated in positive ionisation mode.

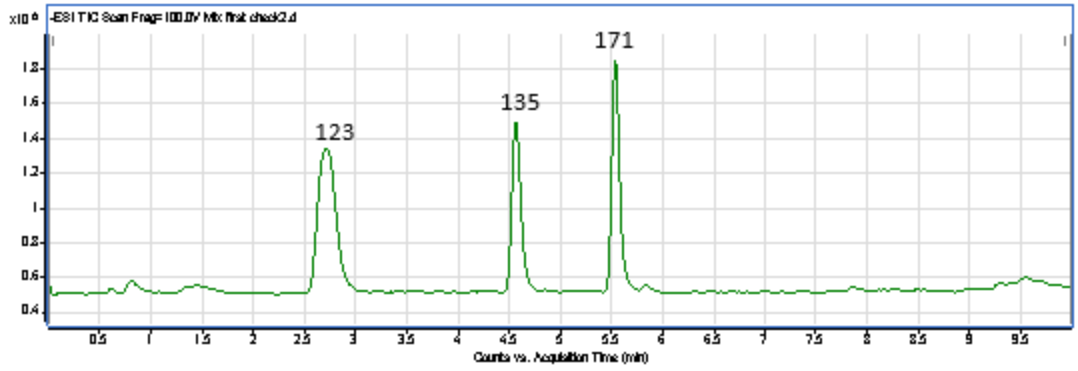


Figure 5.6: TIC to illustrate better retention of compounds with -OCH_3, -*COOH* functional groups. Peak 123 represents methyl catecol, 135 = methyl benzoic acid and 171= naphthoic acid (see Figure 4.1) showing more retention in the column. The ESI was operated in negative ionisation mode.

5.1.4 Effects of column temperature

Column temperature was shown to be a significant factor in the separation and resolution of the peaks. The analyte peak shapes were clustered with poor resolutions when the temperature was increased from 40 until 60°C and was poorer when decreased from 40 until 25°C. These changes were done systematically in 5 to 10 units. Figure 5.7 shows the chromatogram when the column temperature was increased to 50°C.

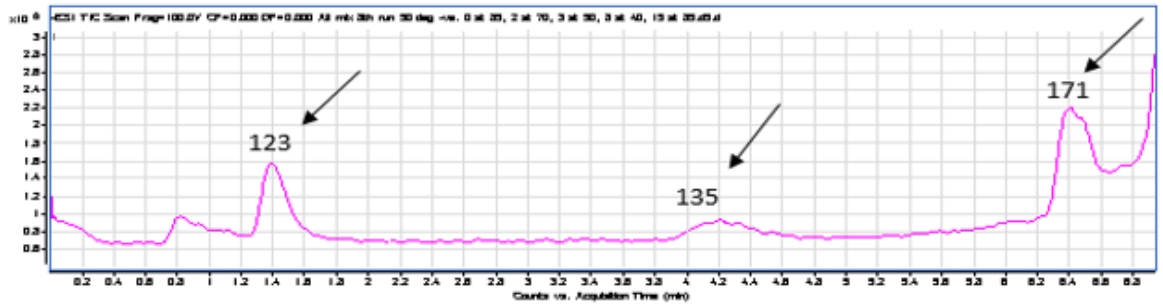


Figure 5.7: Illustrates the TIC at a column temperature of 50°C, showing guaiacol (*m/z* 123), methyl benzoic acid (*m/z* 135) and naphthoic acid (*m/z* 171). The ESI was operated in negative ionisation mode.

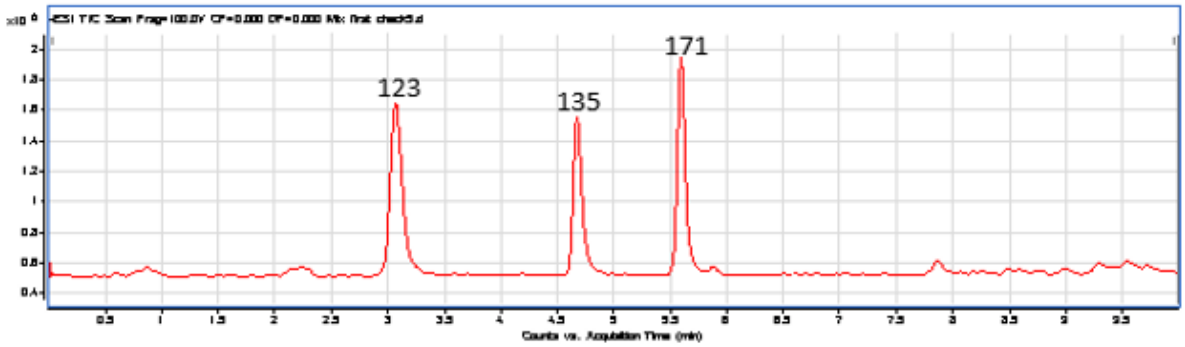


Figure 5.8: Illustrates the TIC at a column temperature of 40°C, showing guaiacol (*m/z* 123), methyl benzoic acid (*m/z* 135) and naphthoic acid (*m/z* 171). The ESI was operated in negative ionisation mode.

Column temperature of 40°C was therefore established as the optimum temperature for the method and this did not change regardless of the analytical columns, this means the temperature in the both columns did not produce any significant change of note.

Table 5.2: The gradient program for the developed method.

Time	**A [H_2O] %**	**B [ACN], 0.1 % acetic acid**
0	85	15
5	50	50
7	45	55
8	40	60
10	85	15

The column temperature was kept at 40°C and the solvent in gradient mode consisted of water [A_2] as the aqueous phase and acetonitrile with 0.1% acetic acid (v/v) in the organic phase (Table 5.2). The initial condition, 85% of [B_1], and 15% of [A_2] was maintained from 0-5 min and then ramped to 50% of [B_1], and 50% of [A_2] then held for 2 mins, changed to 45 and to 40 in 1 min before it was returned back to to 85% in 2 mins. The total analysis time was 10 min, see Table 5.2. Using this gradient condition, reproducible retention times were obtained with an acceptable variation of ±0.5 mins, see Figure 5.9.

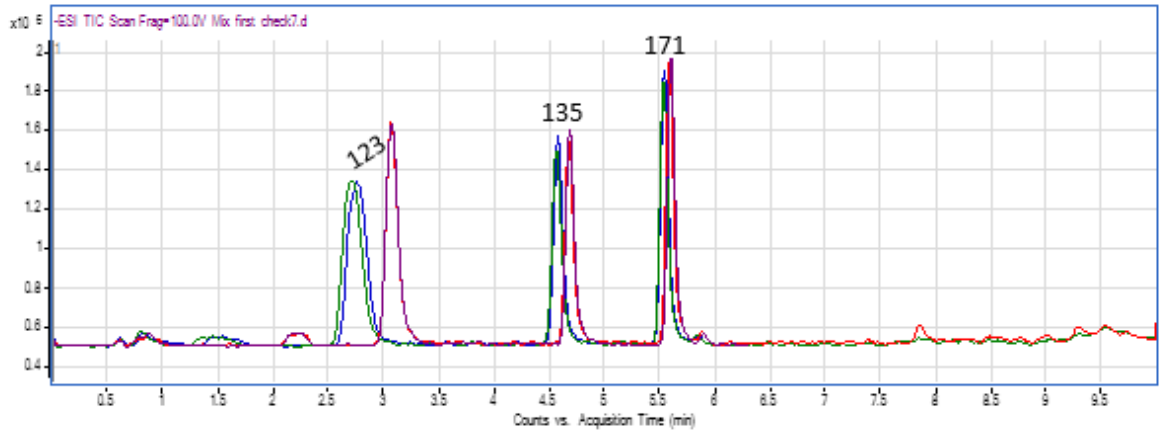


Figure 5.9: TIC showing reproducible retention times of the analytes and approximately ±0.5mins as an acceptable variation between injections. Peak 123 represents methyl catecol, 135 = methyl benzoic acid and 171= naphthoic acid (see Figure 4.1) given increased retention in the column. The ESI was operated in negative ionisation mode.

The flow rate of the HPLC was maintained at 0.3mL/min, and 2µL injection volume was found adequate for this flow rate which produced a pressure between 100-120 bars. The UV detector was maintained at 254nm. Nitrogen was used as nebulizing and drying gas at flow rate of 8L/min at 300°C. The analysis was run in full scan (MS2scan), the scanning operation take 600 times to complete and each individual ions m/z value measured for the fraction of time they elute into the mass analyser from the ESI source. The intensity of peaks within the spectrum are summed to give overall signal intensity against time producing the total ion chromatogram (TIC). The mass scan range was kept from 40-1000 *m/z*, the nebuliser was held at 15 psi, capillary voltage at 4000 V and fragmentor was maintained at 135 to decrease the degree of fragmentaion of the analytes into series of daughter ions. The scan range was found adequate to cover fragment mass of the aromatic groups. The ESI source was operated in negative and positve modes.

Table 5.3: Shows instrumental parameters used in the method.

Parameters	Column 1	Column 2
Column temperature	40°C	-
LC flow rate	0.3ml/min	0.5ml/min
Pressure	100-120 bars	120-200 bars
Ion source	ESI	-
Gas flow	8L/min	-
Scan range	40-1000 *m/z*	-
Capillary voltage	4000 V	
Injection volume	2µl	3 µl
Nebulising gas	N_2 at 15 psi	-
Scan mode	Full MS2scan	-
Scan cycle/sec.	600	-
Fragmentor	135	-
Polarity	$[M-H]^-$ and $[M+H]^+$	-

***(-) in column 2 (Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm), signifies same parameter as in column 1 (ZORBAX SB-C18, RRT; 2.1× 50 mm, 1.8 µm).*

A complete system control, data acquisition and processing were done using the MassHunter Work Station from Agilent, The instrumental parameters are summarised in Table 5.3.

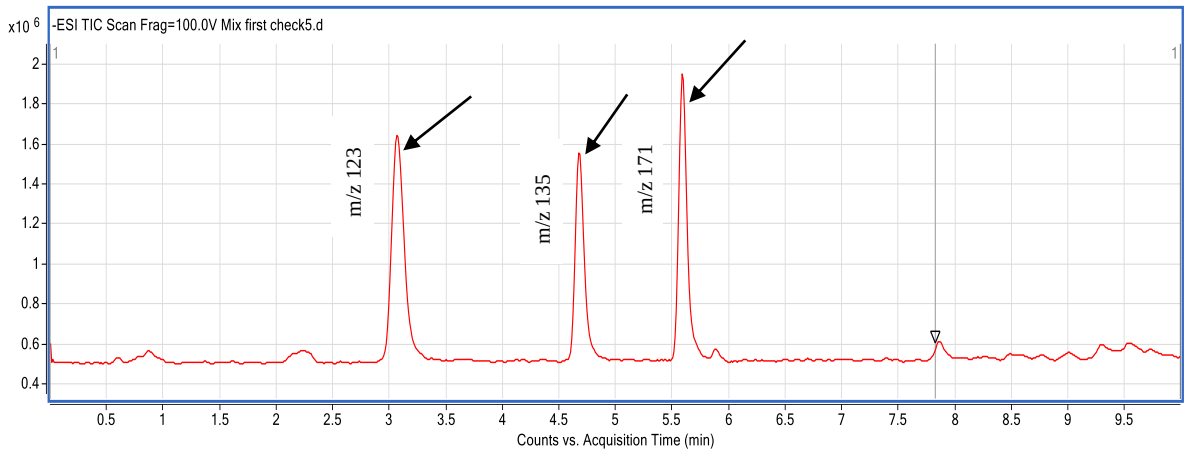


Figure 5.10: TIC of the chosen gradient program of a 2µL injection volume, showing good separation, adequate retention times, great resolution and near clean background noise. Peak 123 represents methyl catecol, 135 = methyl benzoic acid and 171= naphthoic acid (see Figure 4.1). This analysis was run in column 1 in negative ionisation mode.

Reduced injection volume increases the retention of the analytes and also enhances their separation as well as improves the resolution and reproducibility, see Figure 5.10, while increased injection volume upto 3µL results to poor retention and poor separation, see Figure 5.11.

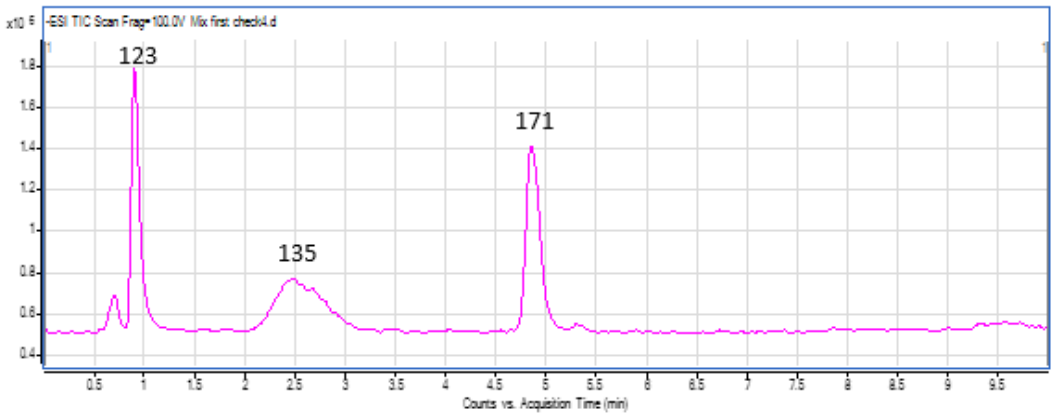


Figure 5.11: The TIC of 3µL injection volume showing poor retention and poor resolution. Peak 123 represents methyl catecol, 135 = methyl benzoic acid and 171= naphthoic acid (see Figure 4.1). The ESI was operated in negative ionisation mode.

In Figure 5.11 the first compound eluted at about 0.8-1 mins when 3µL was injected, however there was a sharp and drastic change when the injection volume was reduced. The first compound eluted at 3.2 mins when the injection volume was 2µL, see Figure 5.12. This observation can be attributed to the tight packing and small internal diameter (1.8µm) of the column. Injecting high volume of sample would overload the column thereby, causing poor retention and forcing the analytes to elute the column earlier.

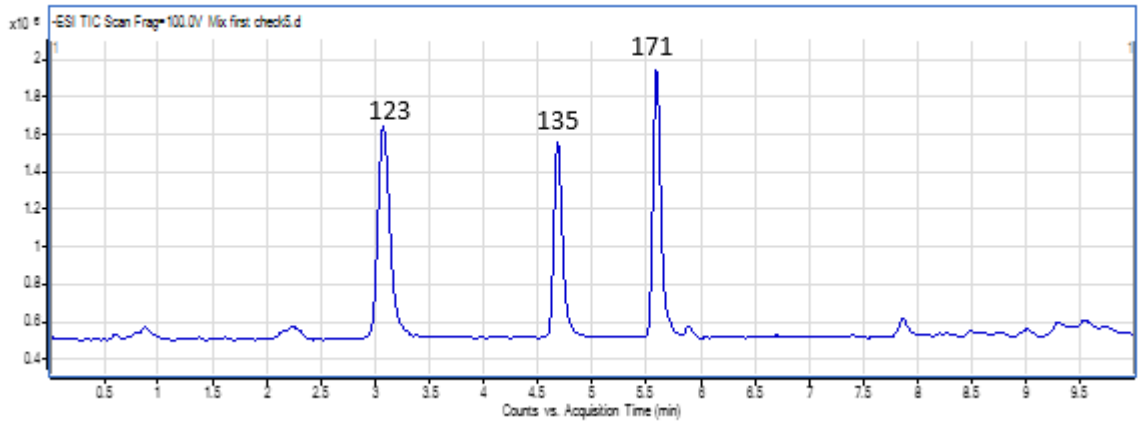


Figure 5.12: The TIC of 2µL injection volume showing good retention and resolution. Peak 123 represents methylcatecol, 135 = methyl benzoic acid and 171= naphthoic acid (see Figure 4.1). The ESI was operated in negative ionisation mode.

Repeated injection while maintaining same conditons, showed some acceptable variation in the retention times of the analyses. See Figure 5.12, in this chromatogram the first compound eluted at approximately 3.2 mins while in and Figure 5.13 the same compound eluted at about 2.7mins. This is an acceptable variation of ± 0.5mins on a repeated injection.

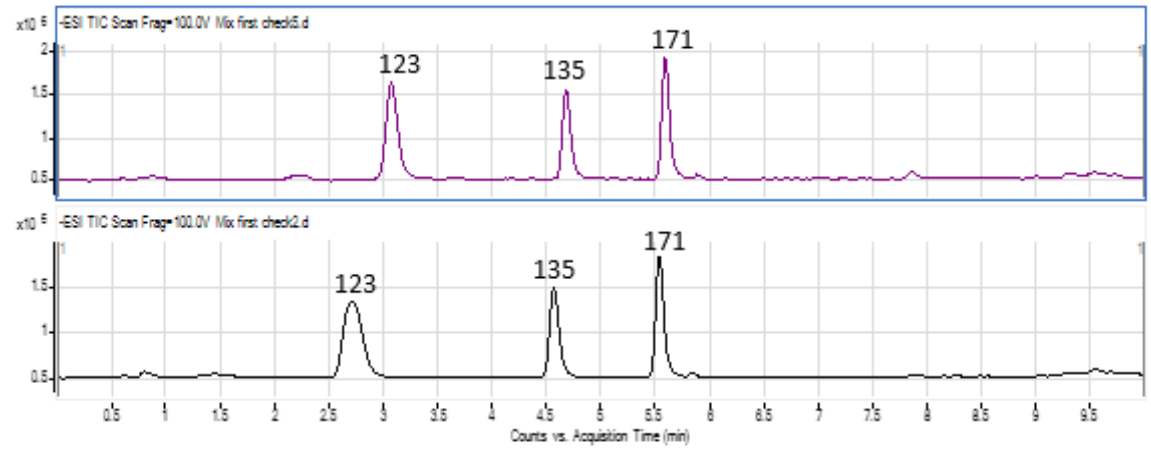


Figure 5.13: Overlay TIC showing variation in retention.

Figure 5.14-5.24 shows chromatograms and corresponding spectrum of the standard analytes used in the method development. The spectrum show the molecular masses detected by the MS instrument and the structures of the molecular ion of the compunds are drawn next to the peaks in chromatograms.

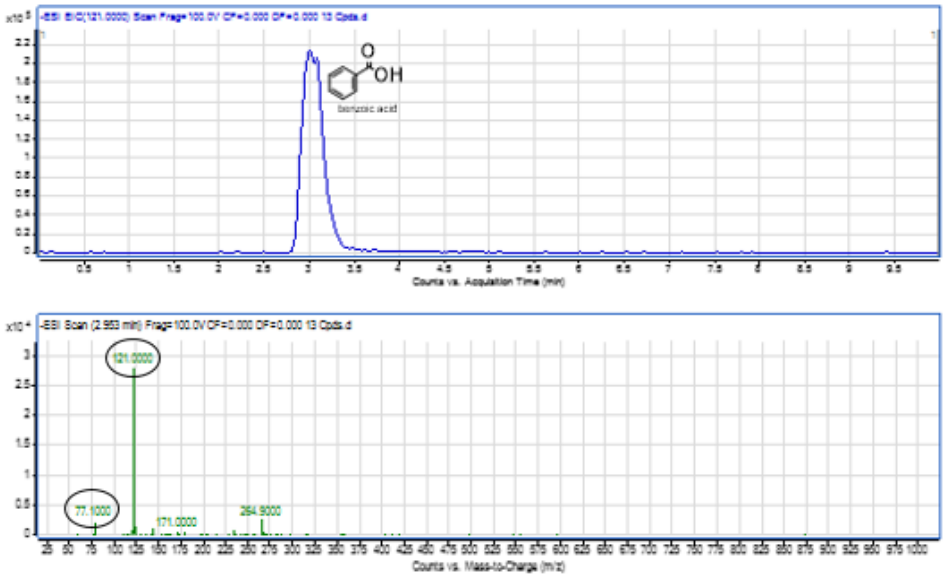


Figure 5.14: TIC and the corresponding spectrum of the peak maximum of benzoic acid (*m/z* 121) showing fragment of *m/z* 77 (M-45). The ESI was operated in negative ionisation mode and each mass of interest is circled in black in the mass spectrum.

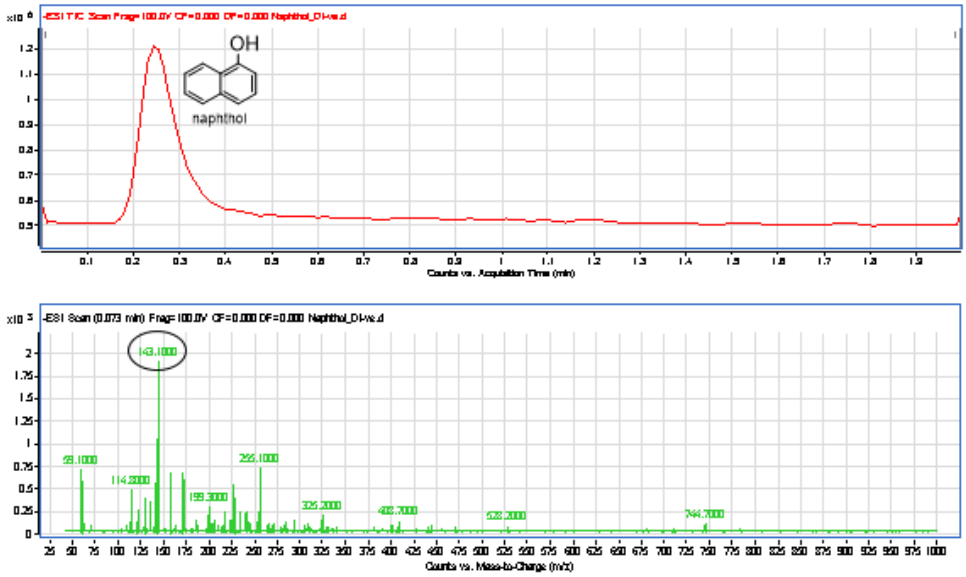


Figure 5.15: TIC and the corresponding spectrum. The mass of interest (peak maximum of naphthol, *m/z* 143) is circled in black in the mass spectrum. The ESI was operated in negative ionisation mode.

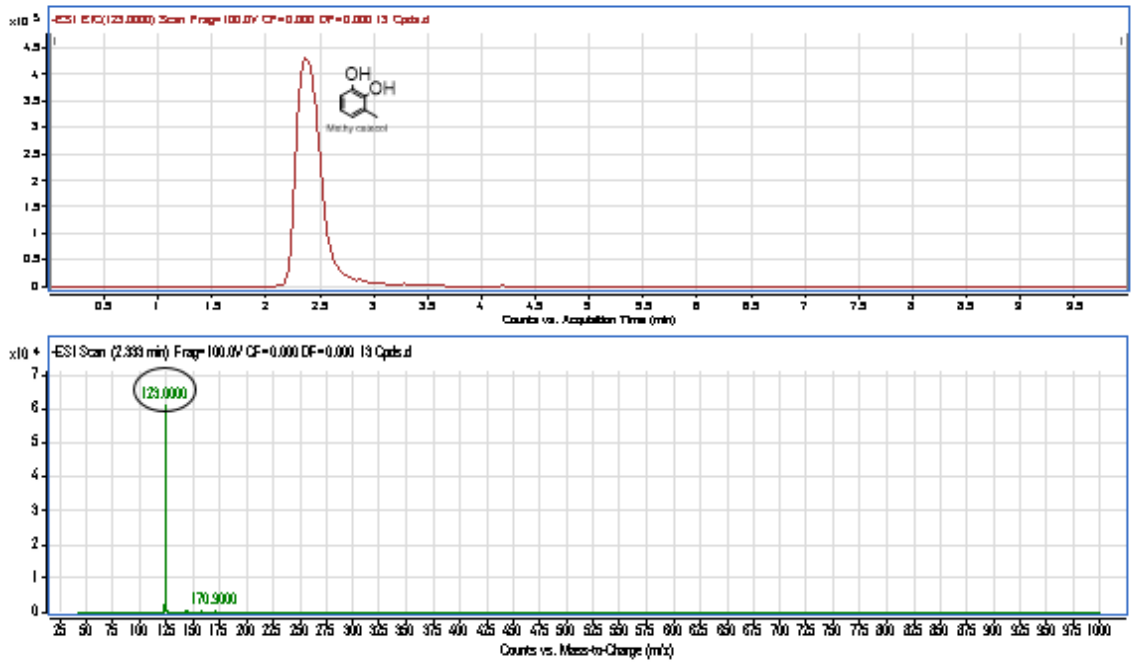


Figure 5.16: TIC and the corresponding spectrum. The mass of interest (peak maximum of methyl catecol, *m/z* 123) is circled in black in the mass spectrum. The ESI was operated in negative ionisation mode.

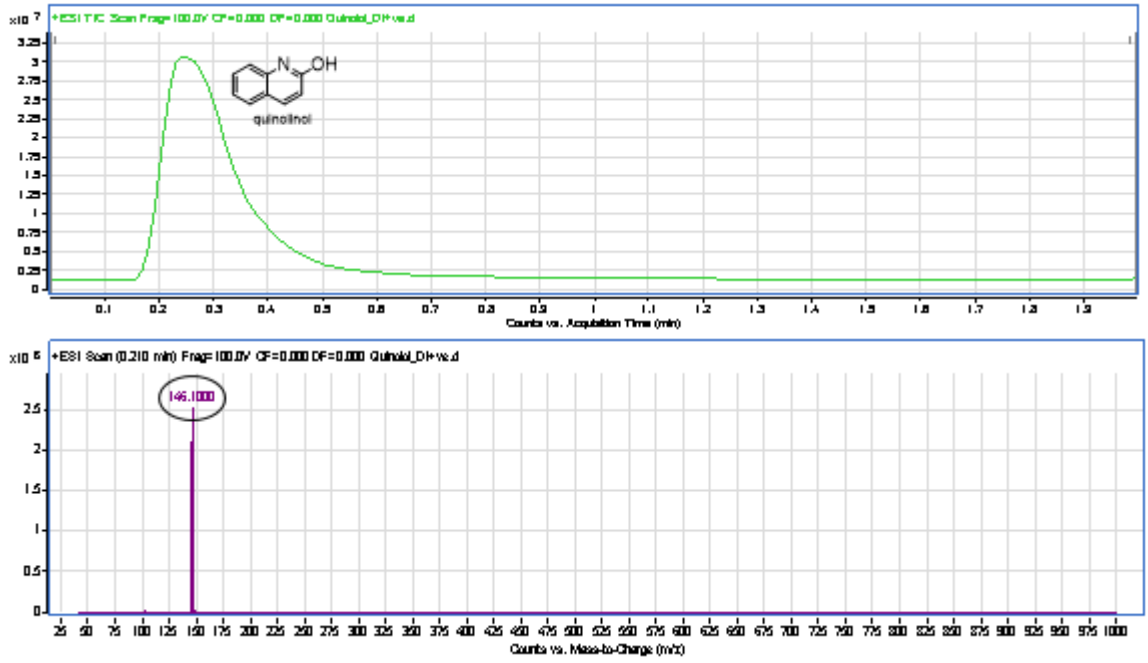


Figure 5.17: TIC and the corresponding spectrum. The mass of interest (peak maximum of quinolinol, *m/z* 146) is circled in black in the mass spectrum. The ESI was operated in negative ionisation mode.

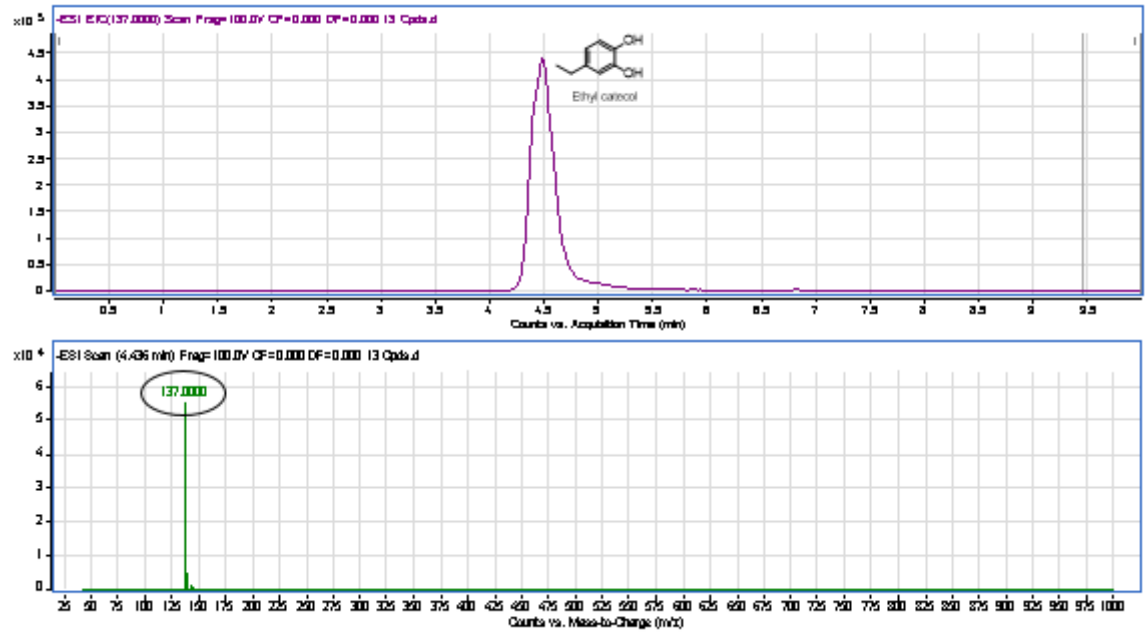


Figure 5.18: TIC and the corresponding spectrum. The mass of interest (peak maximum of ethyl catecol, *m/z* 137) is circled in black in the mass spectrum. The ESI was operated in negative ionisation mode.

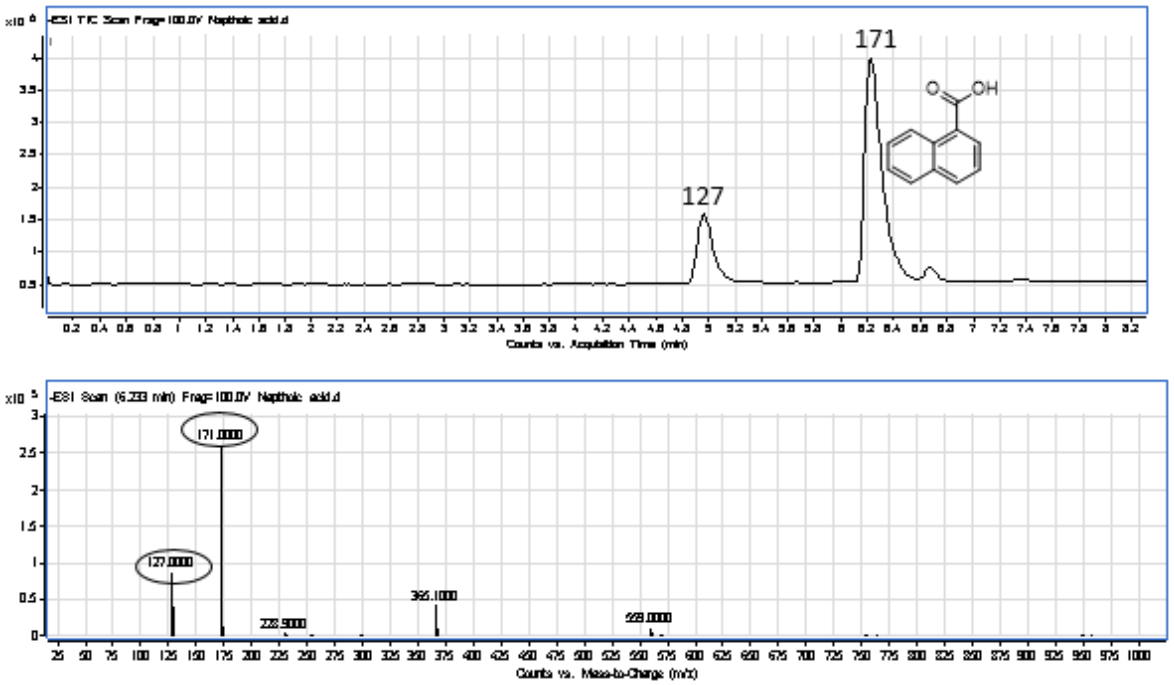


Figure 5.19: TIC of the peak maximum of naphthoic acid (*m/z* 171) showing the corresponding spectrum and likely decomposition of 127 (M-45). The ESI was operated in negative ionisation mode and each mass of interest is circled in black in the mass spectrum.

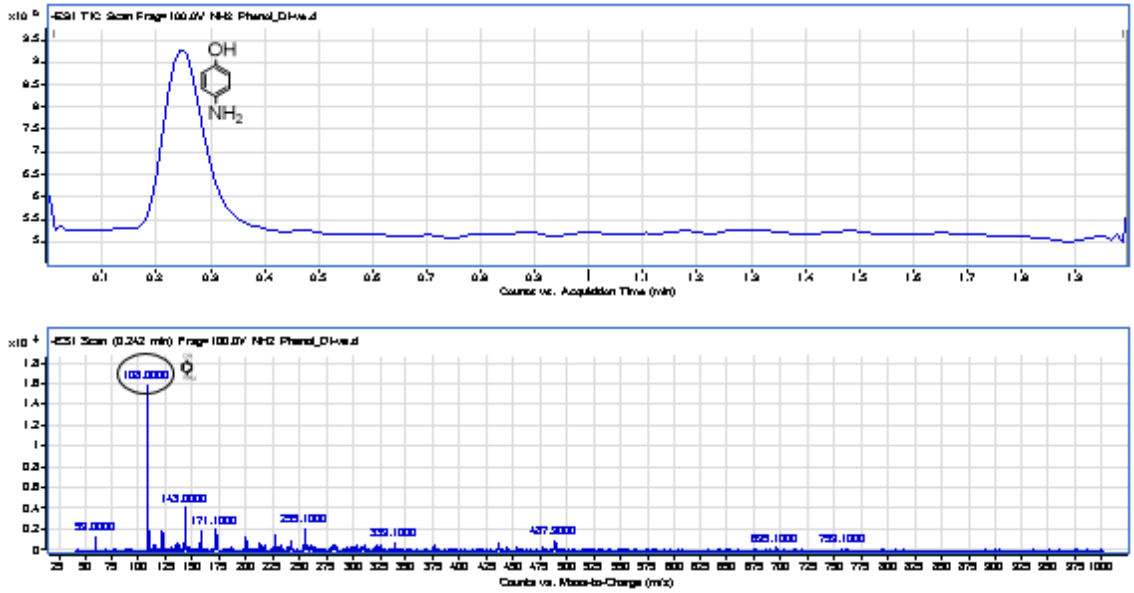


Figure 5.20: TIC and the corresponding spectrum. The mass of interest (peak maximum of amino phenol, *m/z* 108) is circled in black in the mass spectrum. The ESI was operated in negative ionisation mode.

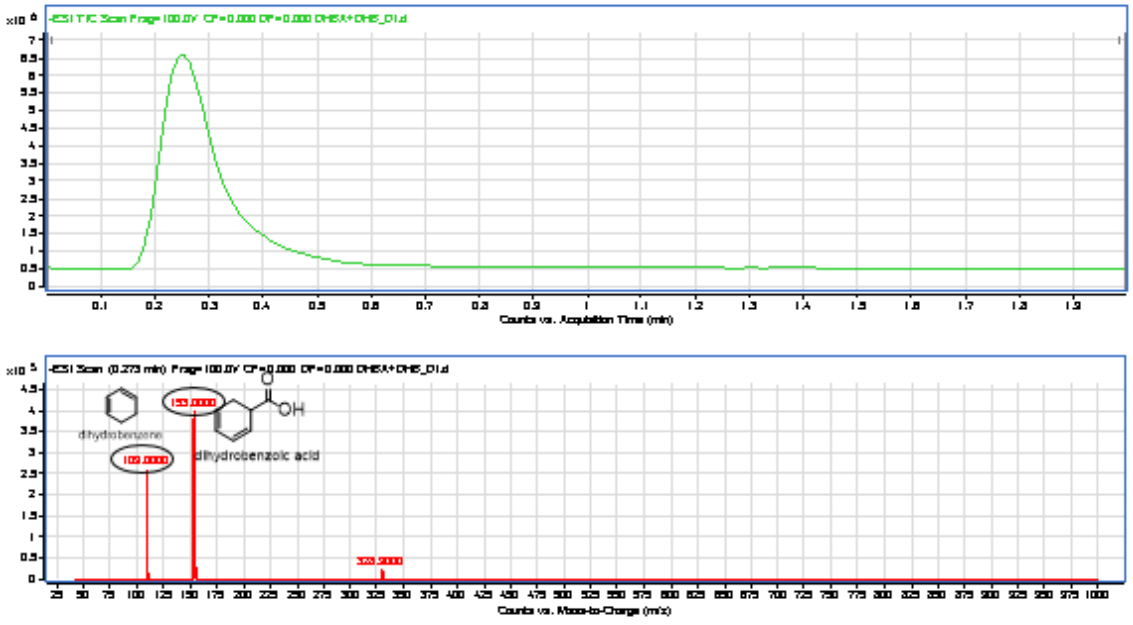


Figure 5.21: TIC from DI-ESI and the corresponding mass spectrum of the sum of the resolved peak of dihydroxybenzene (*m/z* 109) and dihydroxybenzoic acid (*m/z* 153). The ESI was operated in negative ionisation mode and each mass of interest is circled in black in the mass spectrum.

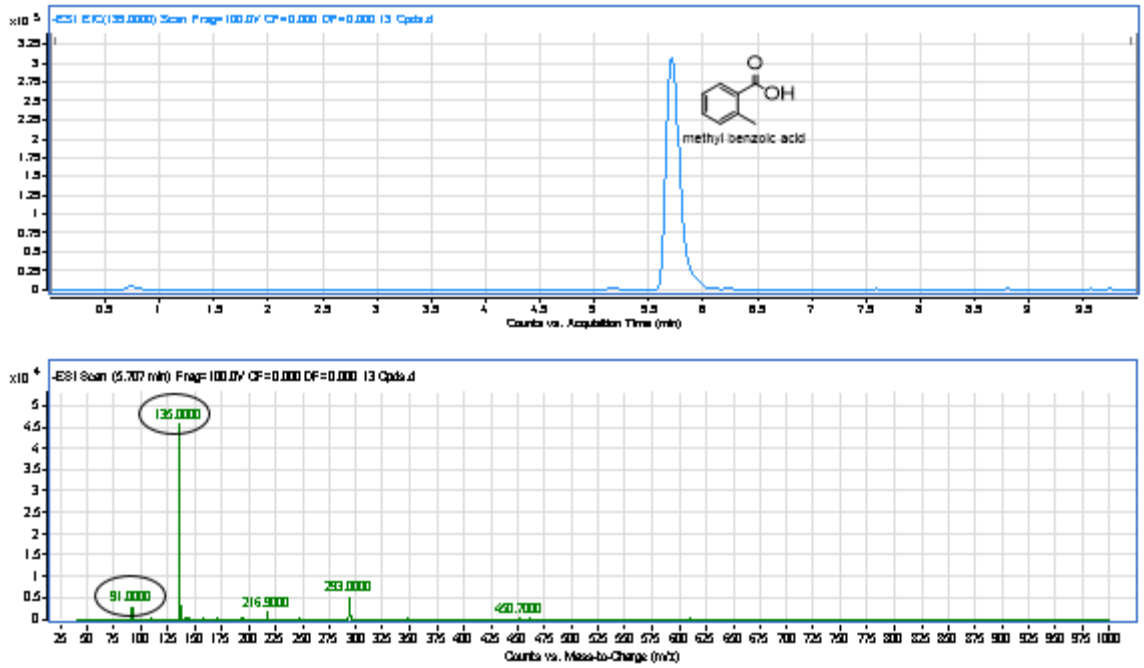


Figure 5.22: The TIC of the peak maximum of methyl benzoic acid (*m/z* 135) and the corresponding spectrum showing *m/z* 91 (M-45). The analysis was done in negative ionisation mode and each mass of interest is circled in black in the mass spectrum.

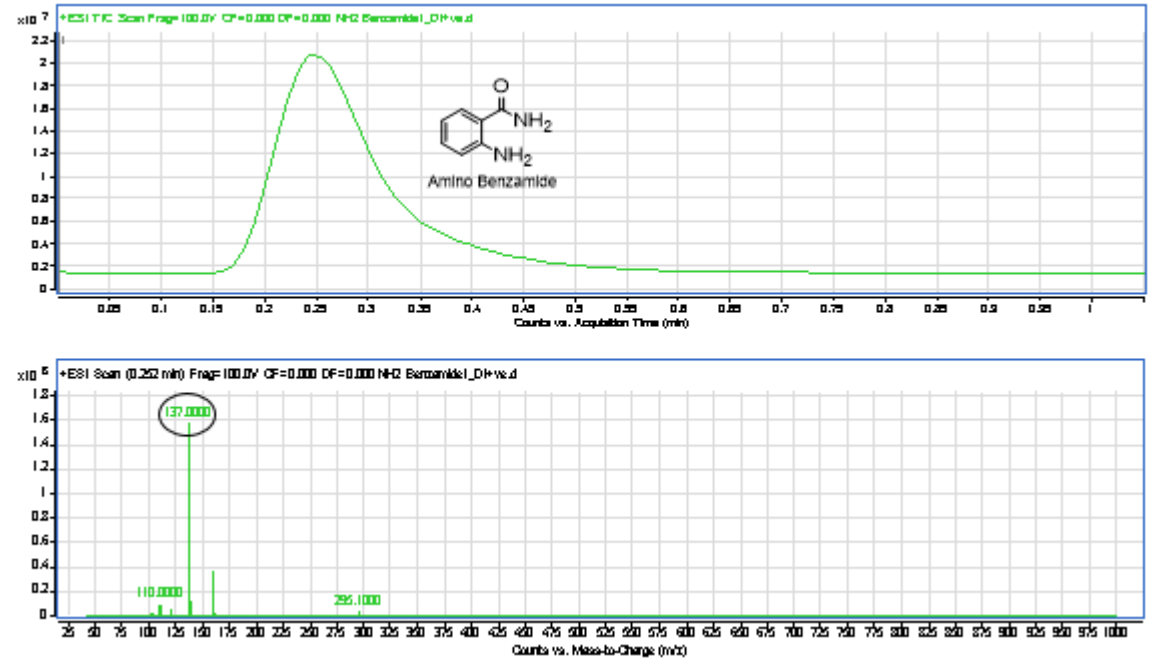


Figure 5.23: The TIC from DI-ESI and the corresponding spectrum. The mass of interest (peak maximum of amino benzamide, *m/z* 137) is circled in black in the mass spectrum. The analysis was done in positive ionisation mode

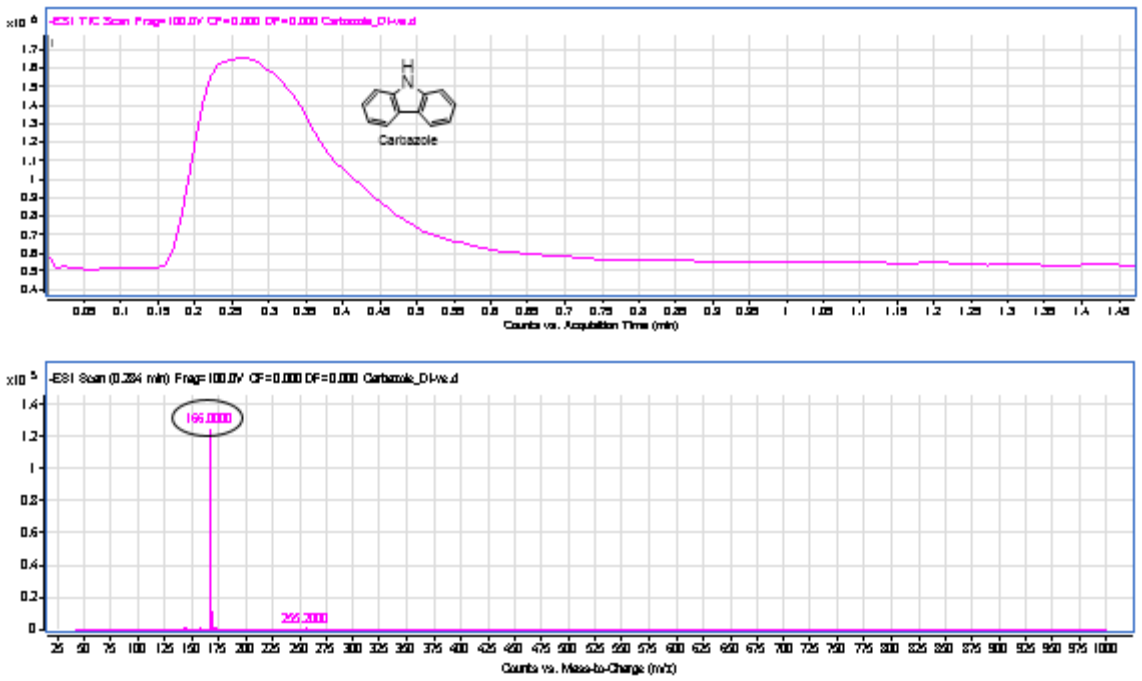


Figure 5.24: TIC from DI-ESI and the corresponding spectrum. The mass of interest (peak maximum of carbazole, *m/z* 166) is circled in black in the mass spectrum. The ESI was operated in negative ionisation mode.

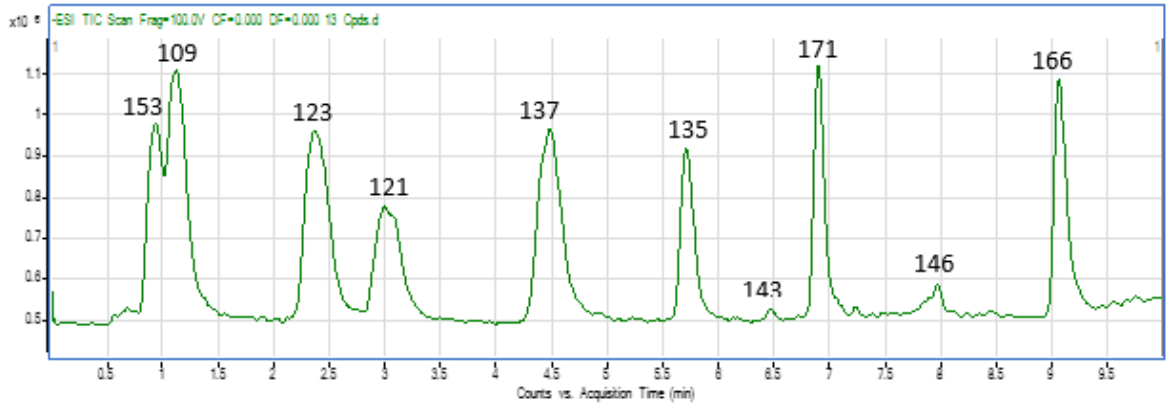


Figure 5.25: LC-TIC of the standards in a mixture, showing well separated peaks and adequate resolution. The *m/z* 153 represents dihydroxybenzoic acid (DHBA), 109 represents dihydroxybenzene (DHB), 123= methyl catecol, 121=benzoic acid, 137= ethyl catecol, 135= methyl benzoic acid, 143= naphthol, 171= naphthoic acid, 146= quinolinol, 166= carbazole. The analysis was done in column 1 and the ESI was operated in negative ionisation mode. The approximate retention times of each compound is listed in Table 5.4 below.

Table 5.4: List of approximate retention times of the standard compounds in the mixture.

Standard compounds	***m/z***	**Approximate retention times (mins)**
Dihydroxybenzoic acid (DHBA)	153	0.9
Dihydroxybenzene (DHB)	109	1.2
Methyl catecol	123	2.4
Benzoic acid	121	3.0
Ethyl catecol	137	4.5
Methyl benzoic acid	135	5.7
Naphthol	143	6.5
Naphthoic acid	171	6.8
Quinolinol	146	8.0
Carbazole	166	9.2

5.1.5 Reproducibility check

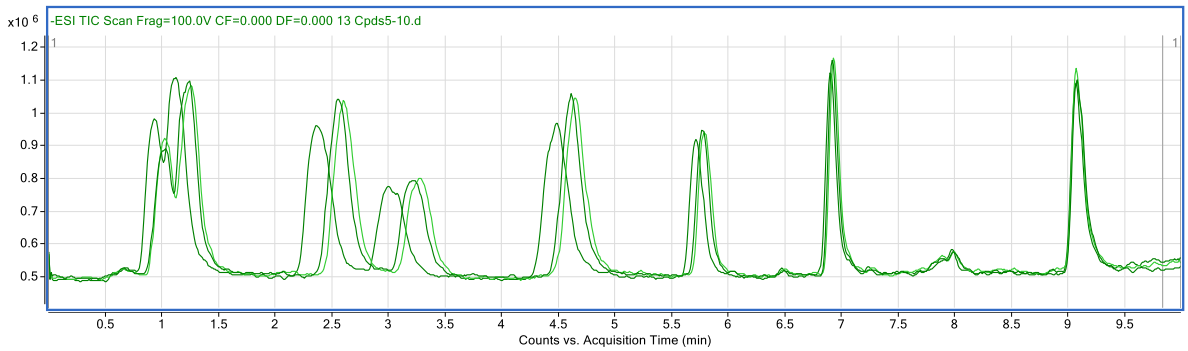


Figure 5.26: LC-MS TIC of three sequential injections to check the reproducibility of the method for the mixture of the standard compounds. The ESI was operated in negative ionisation mode.

The result of Figure 5.26 shows that the method is can separate most of the compounds in the standard mixture and with reproducible retention times. Some of the compounds such as the methyl catecol and guaiacol which have similar molecular masses of 124.13 g/mol and 124.14 g/mol (Table 4.1) respectively seems to be co-eluting from the column and thus the *m/z* of 123 maybe either of the compounds.

Characteristic of the method:

- ✓ Reproducible retention times
- ✓ Good resolution of the peaks
- ✓ Clear identification of the target masses
- ✓ No background noise
- ✓ Minimal ion suppression of the target masses

6.0 METHOD APPLICATION

6.1 Fingerprinting of petroleum and lignin based bio-oils

The fingerprinting approach was performed by dissolving the aliquots from the whole oil (4.8) in acetonitrile. The concentration of the samples injected into the mass instrument was 200µg/ml. The samples were submitted to both direct ESI-MS and LC-ESI-MS and analysed in negative mode polarity. Throughout the rest of this thesis, all analyses were done in negative ionisation, unless where otherwise stated.

6.1.1 Fingerprinting of crude oils

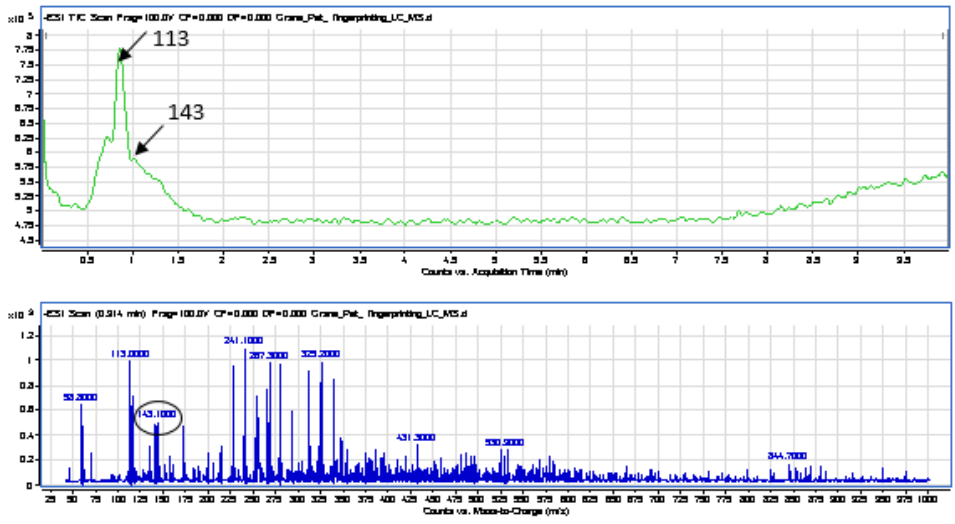


Figure 6.1: TIC from LC-MS of non-fractionated *Grane* crude oil with the corresponding mass spectrum. The *m/z* 143 (naphthol) circled in black is an analogue to the standard used during the method development, the mass is around the shoulder of the resolved peak as indicated by arrows.

Major molecular masses in Figure *6.1*							
113	143	241	287	323	431	530	844

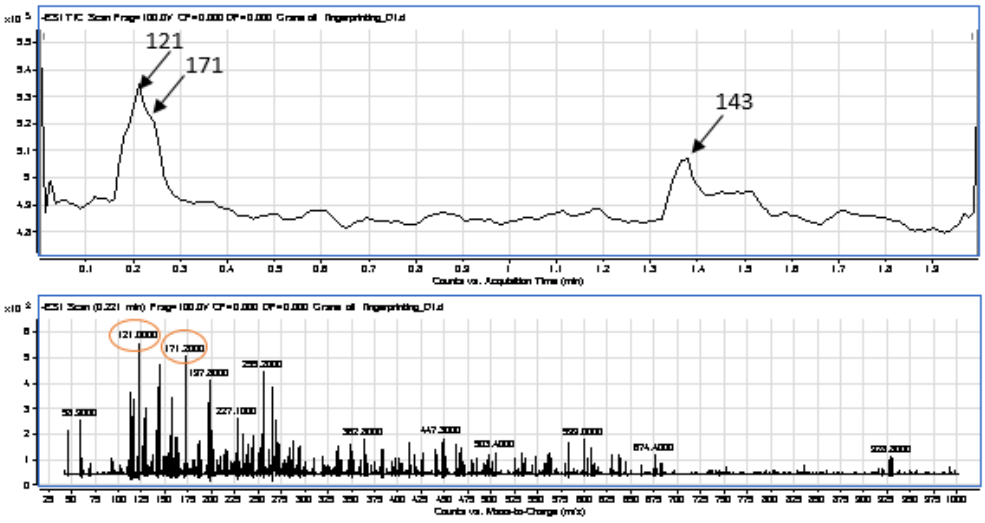


Figure 6.2: TIC from ESI chromatogram of non-fractionated *Grane* crude oil with the corresponding mass spectrum. The *m/z* 121 (benzoic acid) and *m/z* 171 (naphthoic acid) circled in orange are some analogues to the standard used during the method development. Some of the masses are indicated with arrows in the region they are found in the resolved peaks.

Major molecular mass in Figure *6.2*									
121	171	197	227	270	382	447	503	523	847

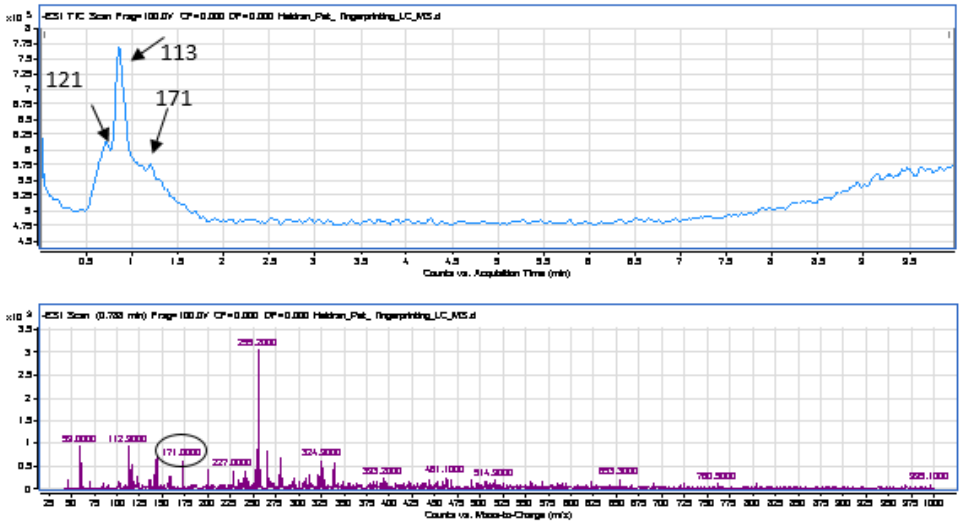


Figure 6.3: TIC from LC-MS of non-fractionated H*eidrun* (biodegraded) crude oil with the corresponding mass spectrum. The *m/z* 171 (naphthoic acid) circled in black is an analogue to the standard used during the method development. Some of the masses are indicated with arrows in the region they are found in the resolved peaks.

Major molecular mass in Figure *6.3*						
122	171	197	227	293	324	481

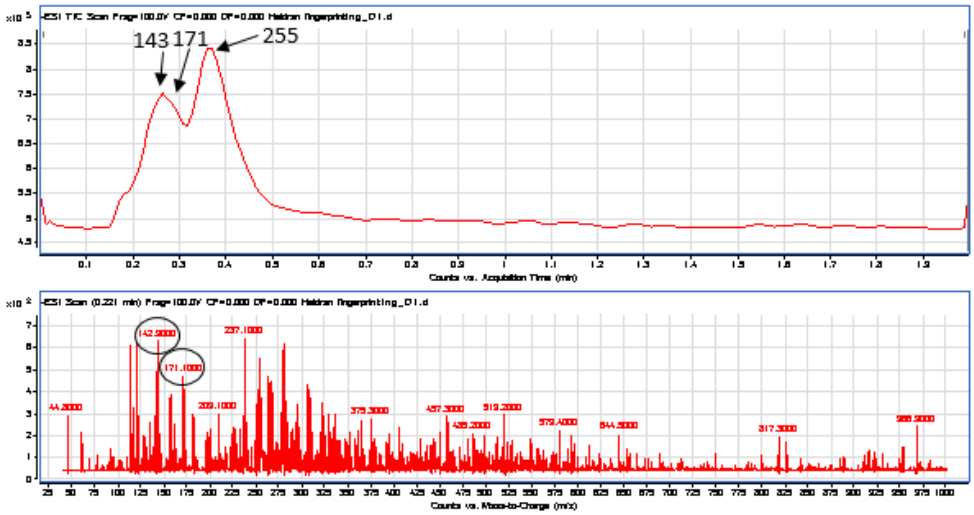


Figure 6.4: TIC from ESI of non-fractionated *Heidrun* (biodegraded) crude oil with the corresponding mass spectrum. The *m/z* 143 (naphthol), *m/z* 171 (naphthoic acid) circled in black is an analogue to the standard used during the method development. Some of the masses are indicated with arrows in the region they are found in the resolved peaks.

Major molecular mass in *Figure 6.4*								
143	171	203	237	373	457	515	544	574

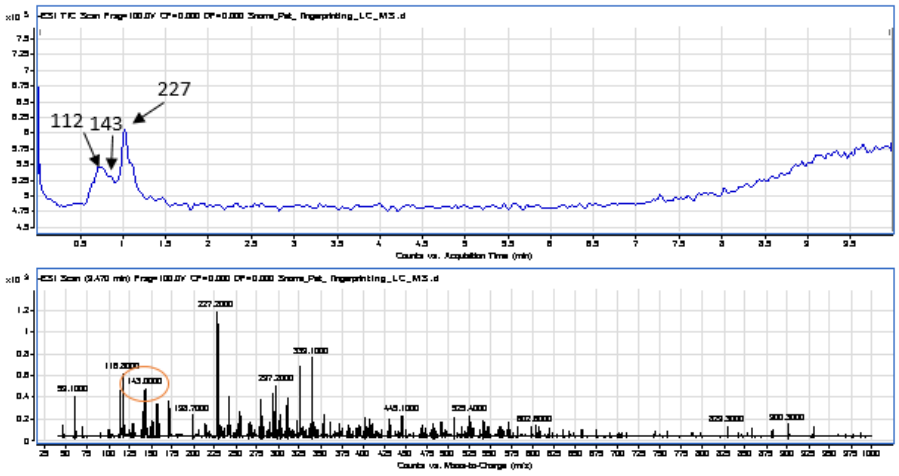


Figure 6.5: TIC from LC-MS of non-fractionated *Snorre* crude oil with the corresponding mass spectrum. The *m/z* 143 (naphthol) circled in orange is an analogue to the standard used during the method development. Some of the masses are indicated with arrows in the region they are found in the resolved peaks.

Major molecular mass in *Figure 6.5*							
116	143	198	227	297	339	445	525

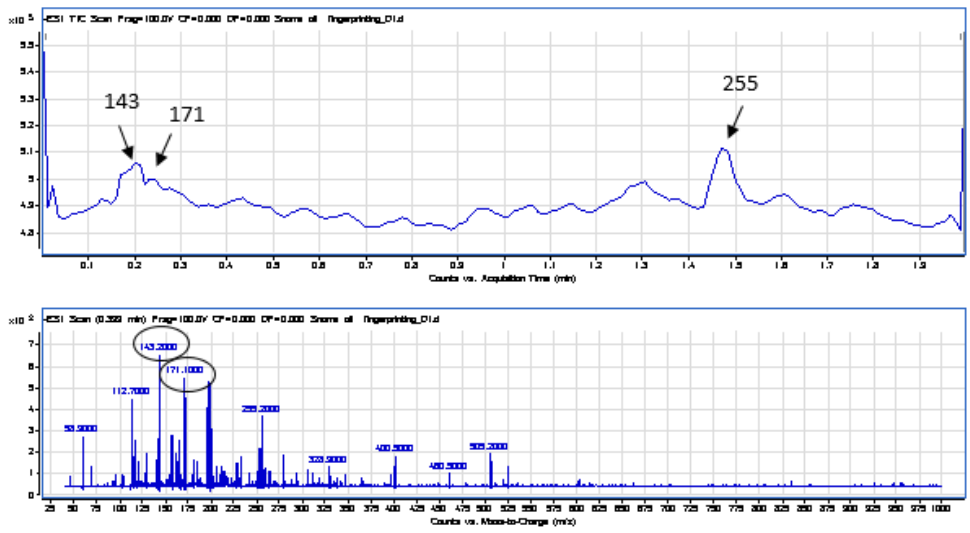


Figure 6.6: TIC from ESI of non-fractionated Sno*rre* crude oil with the corresponding mass spectrum. The *m/z* 143 (naphthol) and *m/z* 171 (naphthoic acid) circled in black are some analogues to the standard used during the method development. Some of the masses are indicated with arrows in the region they are found in the resolved peaks.

Major molecular mass in *Figure 6.6*						
112	143	171	255	400	405	505

6.1.2 Fingerprinting of Bio-oils

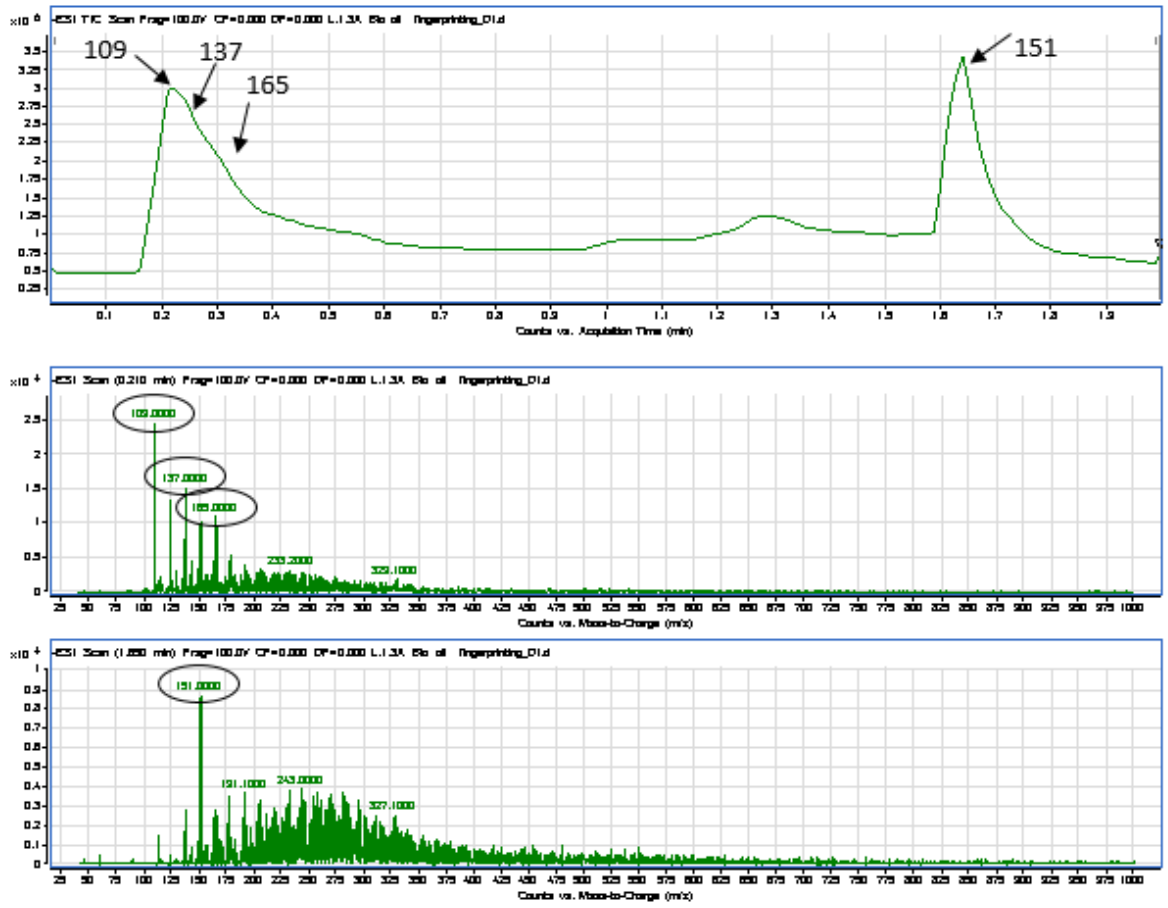


Figure 6.7: TIC from ESI of non-fractionated bio-oil (L.1.3A) with the corresponding mass spectrum showing *m/z* 109 (catecol or dihydroxybenzene), *m/z* 137 (ethyl catecol), *m/z* 153 (dihydroxybenzoic acid). The circled masses in black are some analogues to the standard used during the method development. The *m/z* 151 (could be a Propylbenzene-1,3-diol (Figure 2.8) or other compounds with similar identity). The masses are indicated with arrows in the region they are found in the resolved peaks.

Major molecular mass in *Figure 6.7*							
109	137	151	165	191	233	243	327

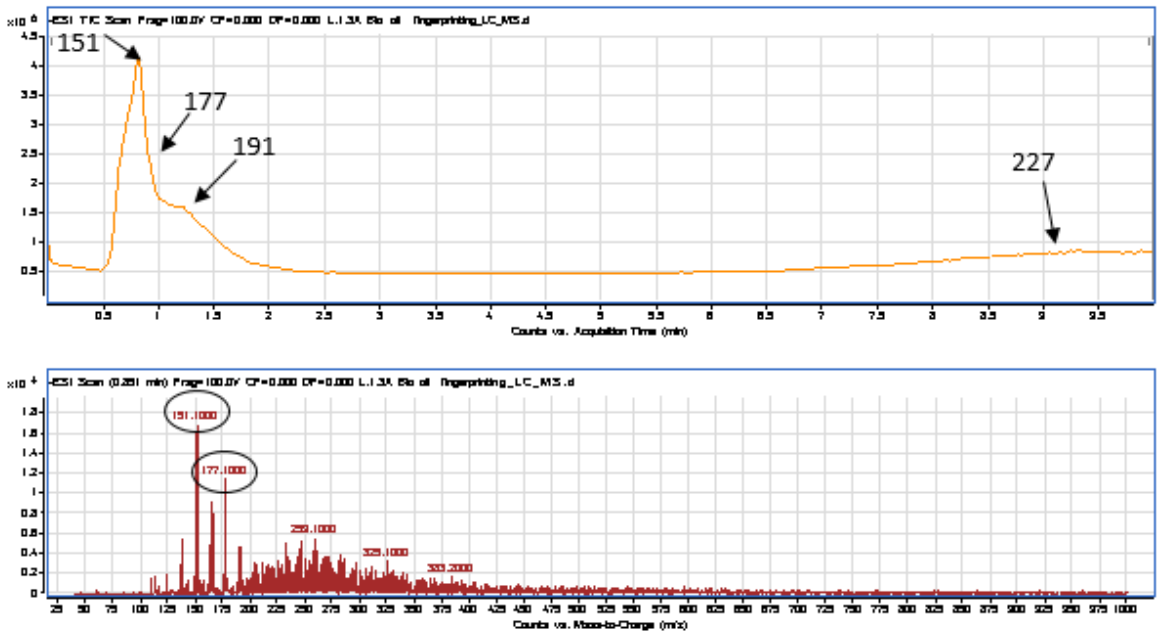


Figure 6.8: TIC from LC-MS of non-fractionated bio oil (L.1.3A) with the corresponding mass spectrum. showing *m/z* 151 (likely, propylbenzene-1,3-diol) and *m/z* 177 (likely methoxy eugenol or other compounds with similar identity) circled in black, see Figure 2.8. These compounds were not used as analogues during the method development. Some of the masses are indicated with arrows in the region they are found in the resolved peaks.

Major molecular mass in *Figure 6.8*						
151	177	191	227	259	325	383

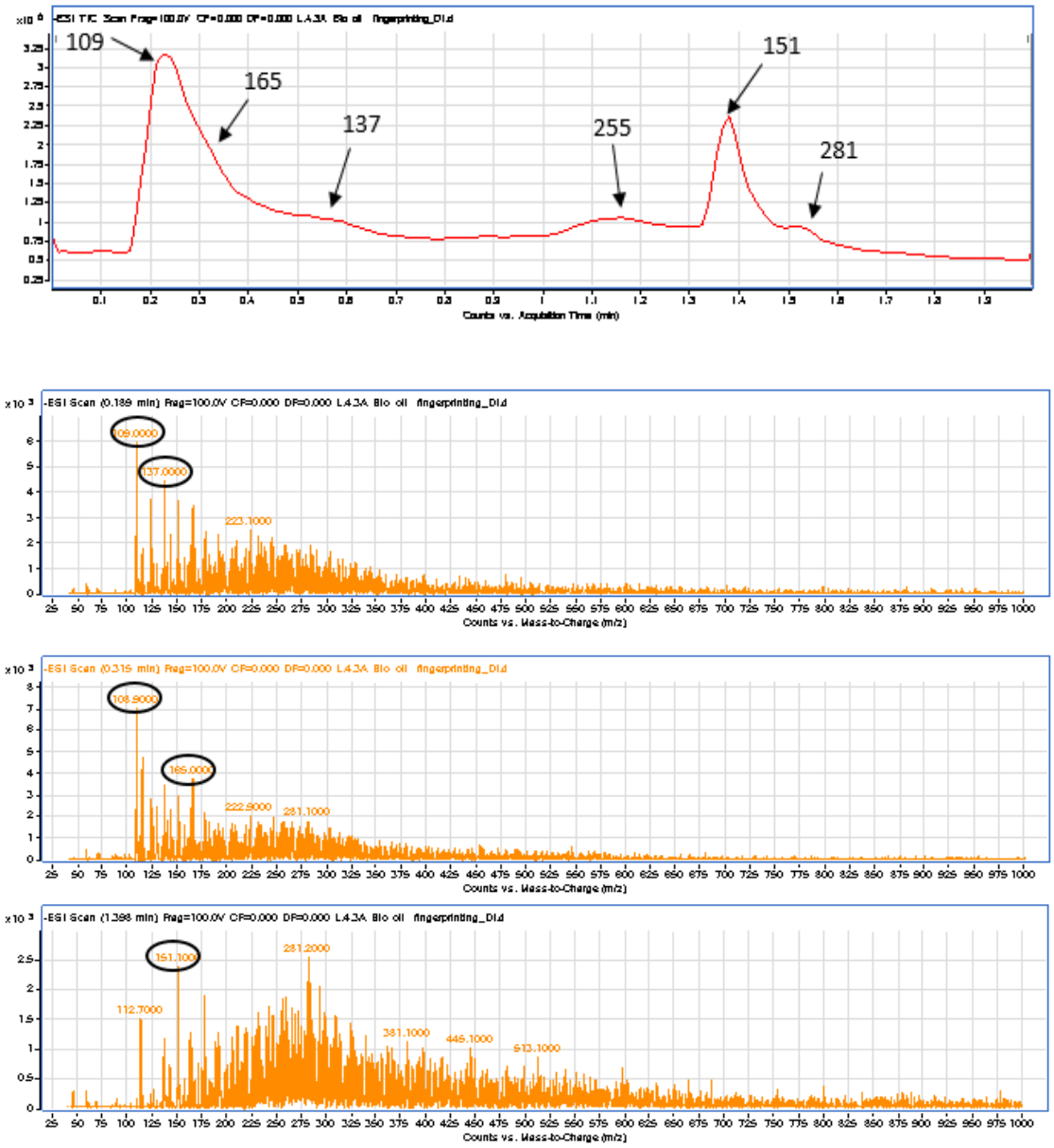


Figure 6.9: TIC from DI-ESI of non-fractionated bio oil (L.4.3A) with the corresponding mass spectra, showing *m/z* 109 (catecol or dihydroxybenzene), *m/z* 137 (ethyl catecol), *m/z* 165 (could be propyl guaiacol or another compound with similar identity) respectively. The *m/z* 151 (likely, propylbenzene-1,3-diol) in black circle, the spectrum, is the peak maximum of the second resolved peak farther right. Some of the masses are indicated with arrows in the region they are found in the resolved peaks.

Major molecular mass in *Figure 6.9*									
109	137	151	112	165	255	281	221	231	381

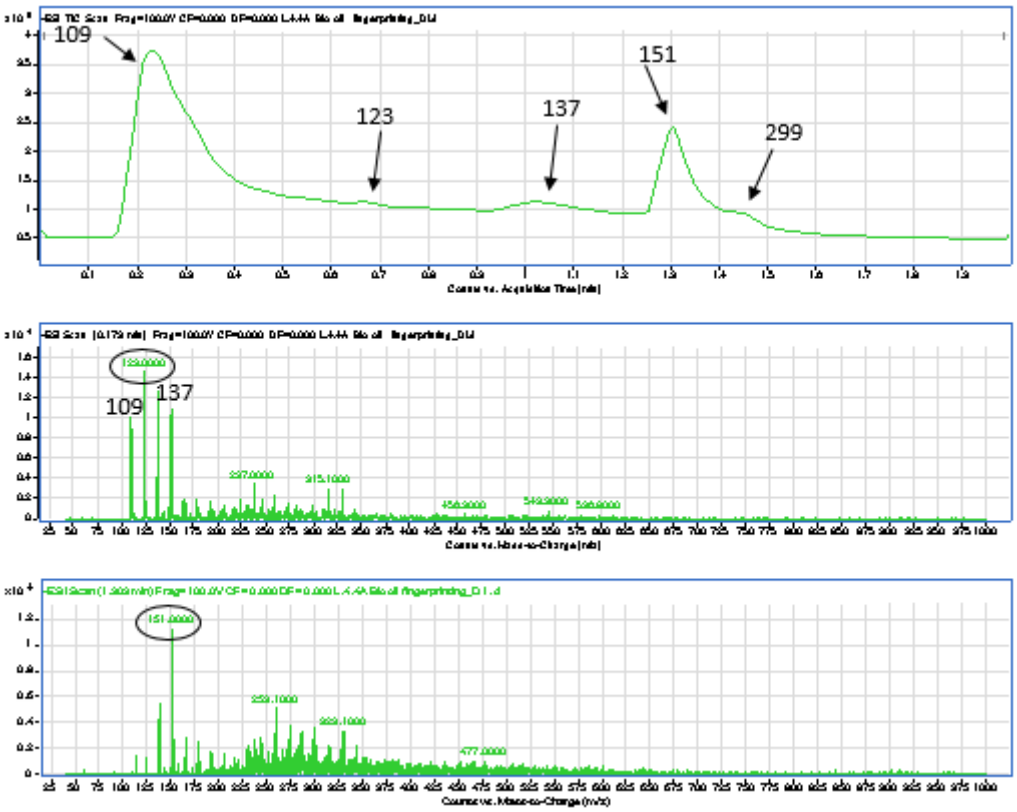


Figure 6.10: TIC from ESI of non-fractionated bio oil (L.4.4A) with the corresponding mass spectrum, showing *m/z* 123 (guaiacol or methyl catecol), *m/z* 109 (catecol or dihydroxybenzene), *m/z* 137 (ethyl catecol), *m/z* 151 (likely, Propylbenzene-1,3-diol) in black circle. Some of the masses are indicated with arrows in the region they are found in the resolved peaks.

Major molecular mass in *Figure 6.10*						
109	123	137	151	299	315	325

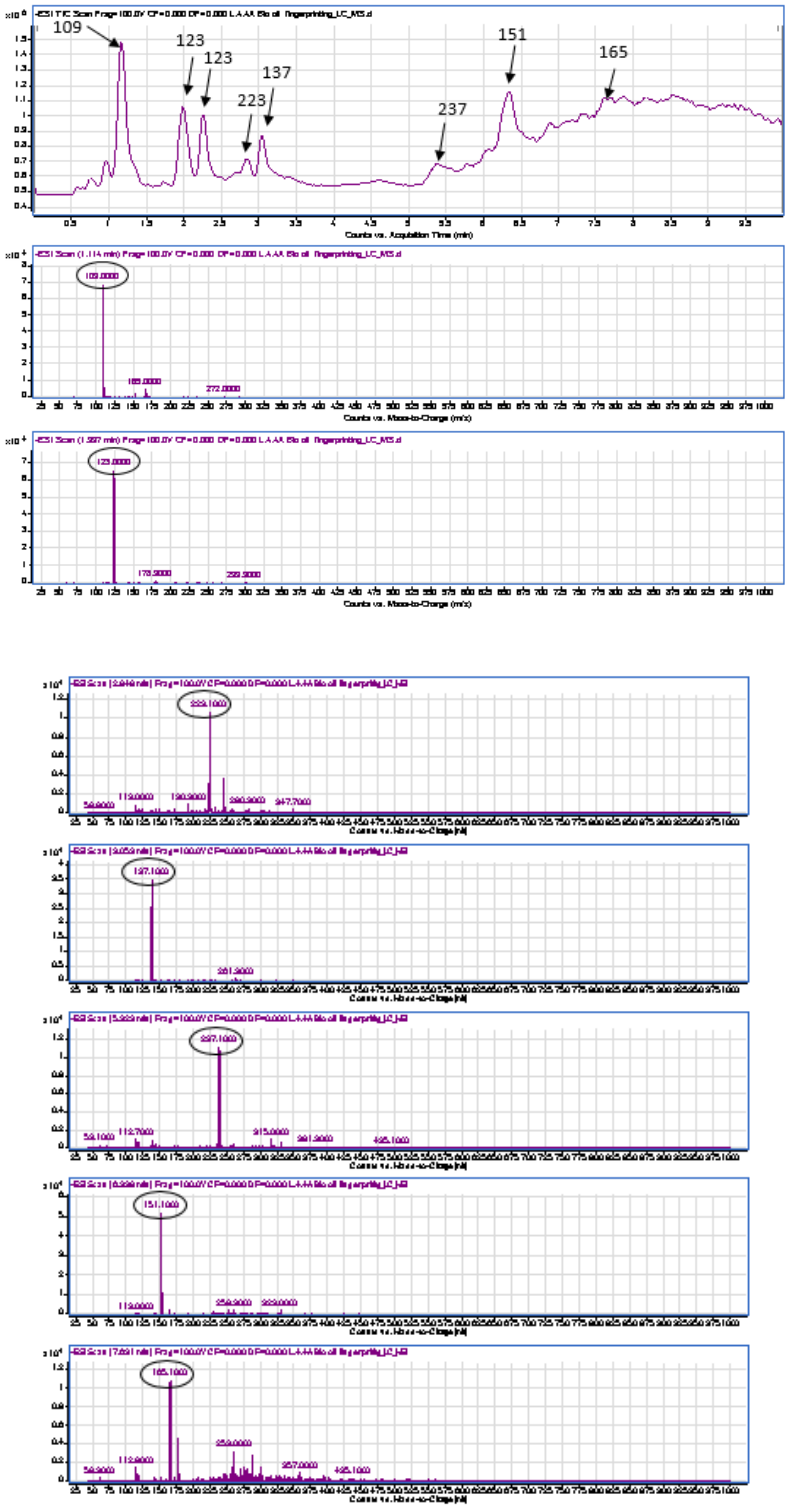


Figure 6.11: TIC from LC-MS of non-fractionated bio oil (L.4.4A) with the corresponding mass spectrum, showing *m/z* 123 (guaiacol or methyl catecol), *m/z* 109 (catecol or dihydroxybenzene), *m/z* 165 (propyl guaiacol) *m/z* 137 (ethyl catecol), *m/z* 151 (propylbenzene-1,3-diol) in black circle. Some of the masses are indicated with arrows in the region they are found in the resolved peaks.

Major molecular mass in *Figure 6.11*						
109	123	137	151	165	233	237

6.2 Characterisation of fractionated oils

Before the LC-MS analysis of the polar fractions, the hydrocarbon components in the oil were removed by washing through Isolute Cyano SPE column from SUPELCO using a solvent containing Hexane: DCM, 90:10 (v/v), see Table 4.3, for the details of this procedure. Injection volume of 2µL of 500 µg/ml was injected into the LC-MS.

6.2.1 Fractionated petroleum oils

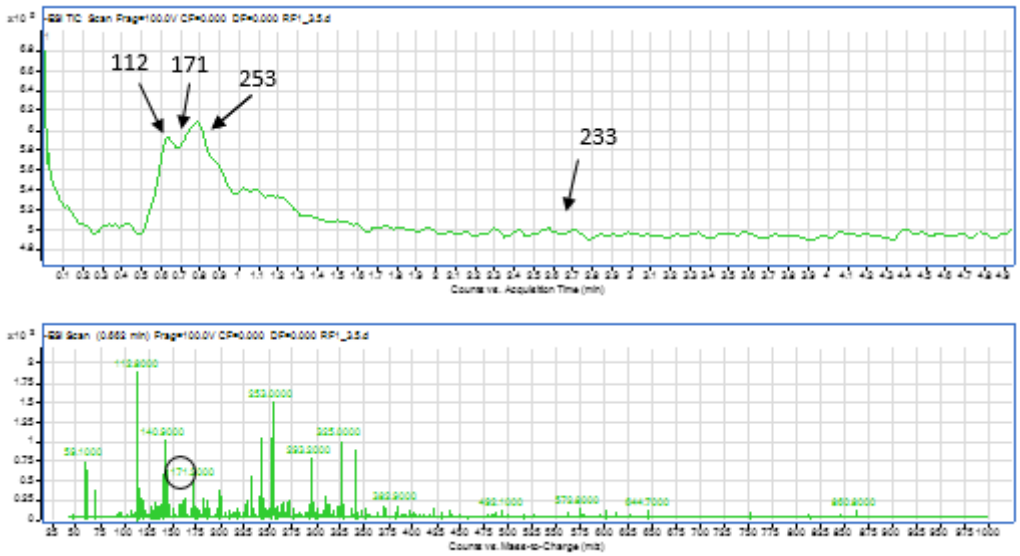


Figure 6.12: TIC from LC-MS of fractionated *Grane* oil with the corresponding mass spectrum. The *m/z* 171 (naphthoic acid) circled in black is an analogue to the standard used during the method development. Some of the masses are indicated with arrows in the region they are found in the resolved peaks.

Major molecular mass in *Figure 6.12*						
112	140	171	233	253	325	332

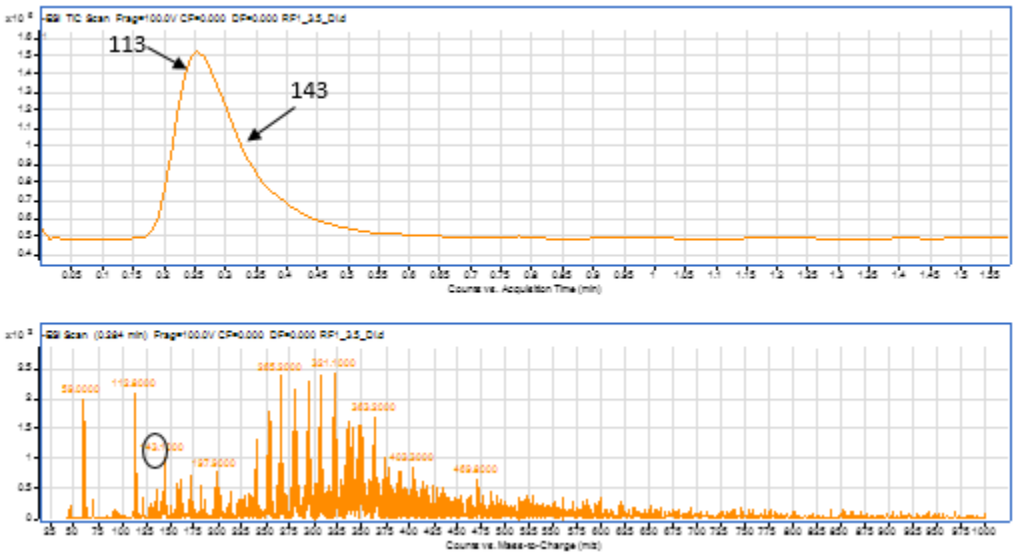


Figure 6.13: TIC from DI-ESI of fractionated *Grane* oil with the corresponding mass spectrum. The *m/z* 143 (naphthol) circled in black is an analogue to the standard used during the method development. Some of the masses are indicated with arrows in the region they are found in the resolved peak.

Major molecular mass in *Figure 6.13*							
112	143	197	293	321	383	403	423

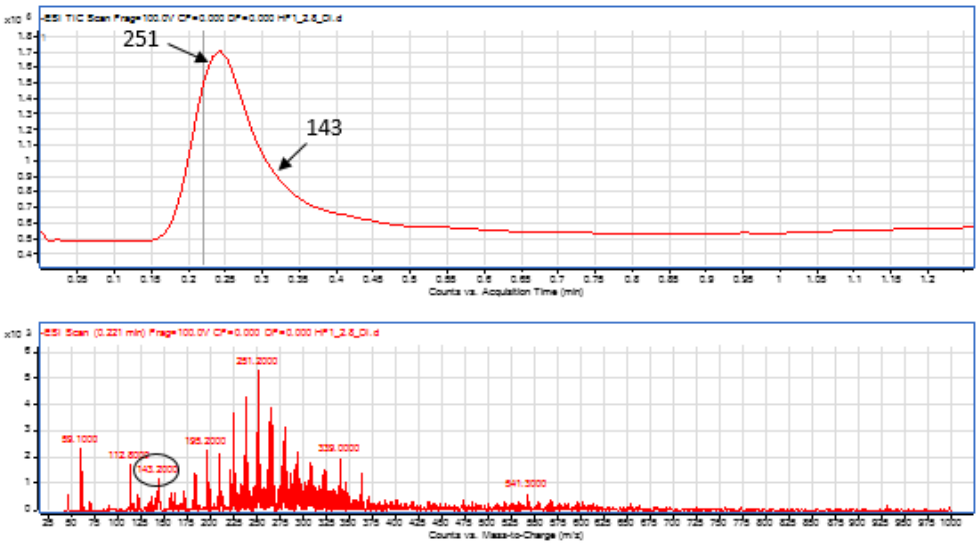


Figure 6.14: TIC from DI-ESI of fractionated *Heidrun* (biodegraded) oil with the corresponding mass spectrum. The *m/z* 143 (naphthol) circled in black is an analogue to the standard used during the method development. Some of the masses are indicated with arrows in the region they are found in the resolved peaks.

Major molecular mass in *Figure 6.14*					
112	143	197	251	291	325

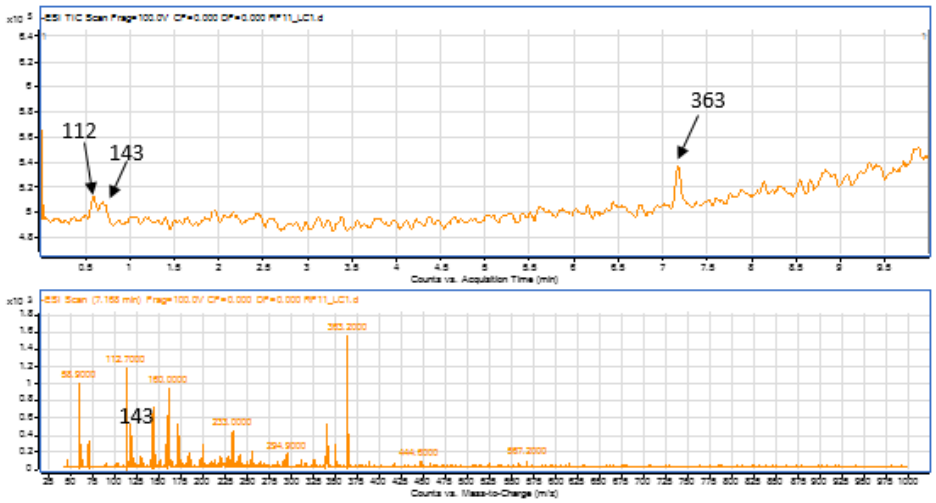


Figure 6.15: TIC from LC-MS of fractionated *Heidrun* (biodegraded) oil with the corresponding mass spectrum from the first peak of the chromatogram. The *m/z* 143 (naphthol) in black is an analogue to the standard used during the method development. Some of the masses are indicated with arrows in the region they are found in the resolved peaks.

Major molecular mass in *Figure 6.15*					
58	112	143	160	233	363

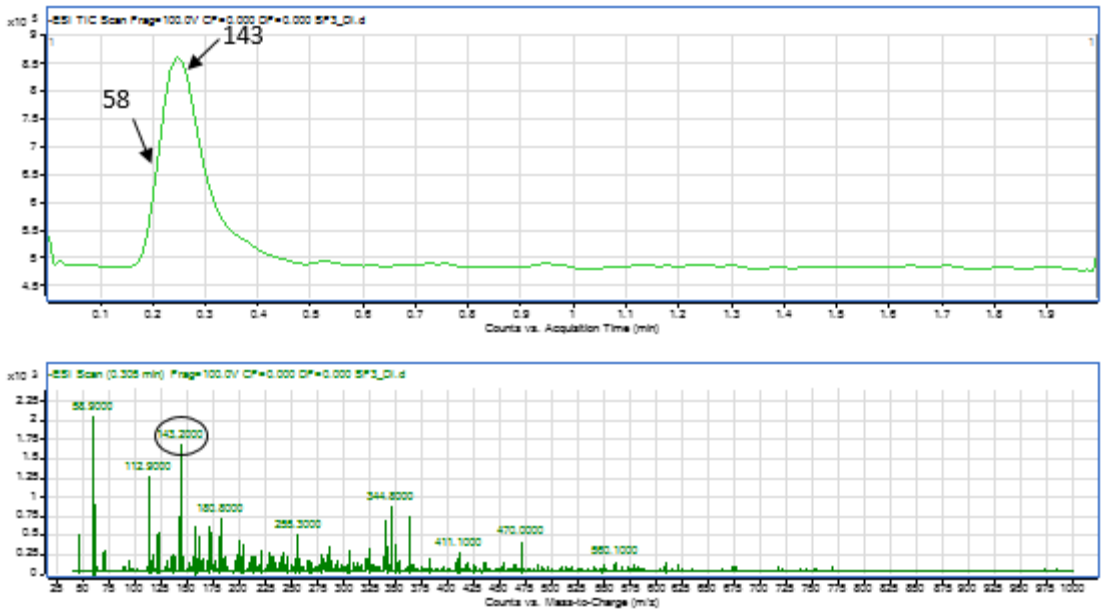


Figure 6.16: TIC from DI-ESI of fractionated *Snorre* oil with the corresponding mass spectrum. The *m/z* 143 (naphthol) circled in black is an analogue to the standard used during the method development. Some of the masses are indicated with arrows in the region they are found in the resolved peak.

Major molecular mass in *Figure 6.16*							
58	112	143	183	255	344	411	470

6.2.2 Fractionated Bio-oils

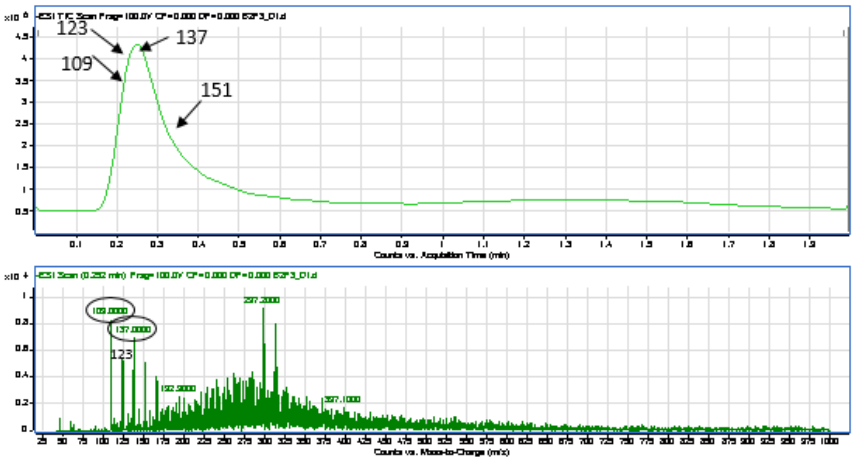


Figure 6.17: TIC from DI-ESI of lignin bio-oil (AS.II.3.2) and the corresponding spectrum. Showing *m/z* 109 (DHB or catecol), *m/z* 123 (methyl catecol or guaiacol), *m/z* 137 (ethyl catecol), and *m/z* 151 (likely, propylbenzene-1,3-diol) Some of the masses are indicated with arrows in the region they are found in the resolved peak.

Major molecular mass in *Figure 6.17*Figure *6.15*					
109	123	137	151	255	297

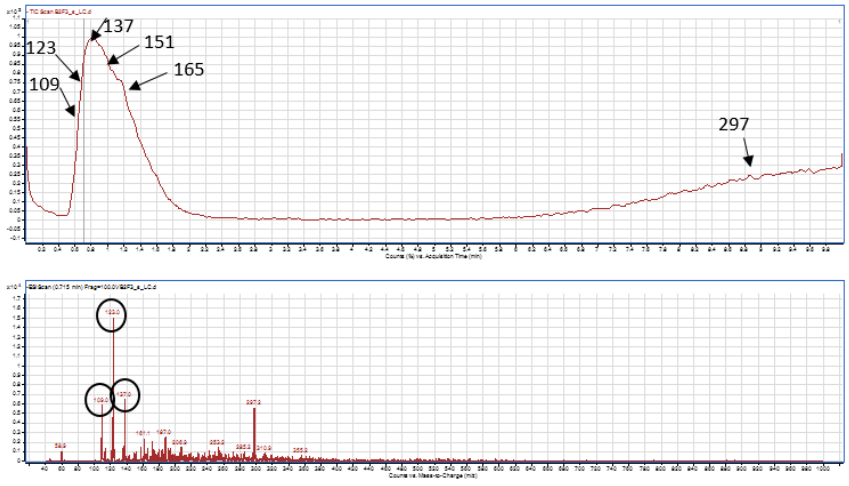


Figure 6.18: TIC from LC-MS of lignin bio-oil (AS.II.E.3.2) and the corresponding spectrum. Showing m/z 109 (DHB or catecol), *m/z* 123 (methyl catecol or guaiacol), *m/z* 137 (ethyl catecol), *m/z* 151 (propylbenzene-1,3-diol), *m/z* 165 (could be a propyl guaiacol or another compound with similar identity) in black circles. Some of the masses are indicated with arrows in the region they are found in the resolved peak.

Major molecular mass in *Figure 6.18*								
109	123	137	165	206	253	285	297	310

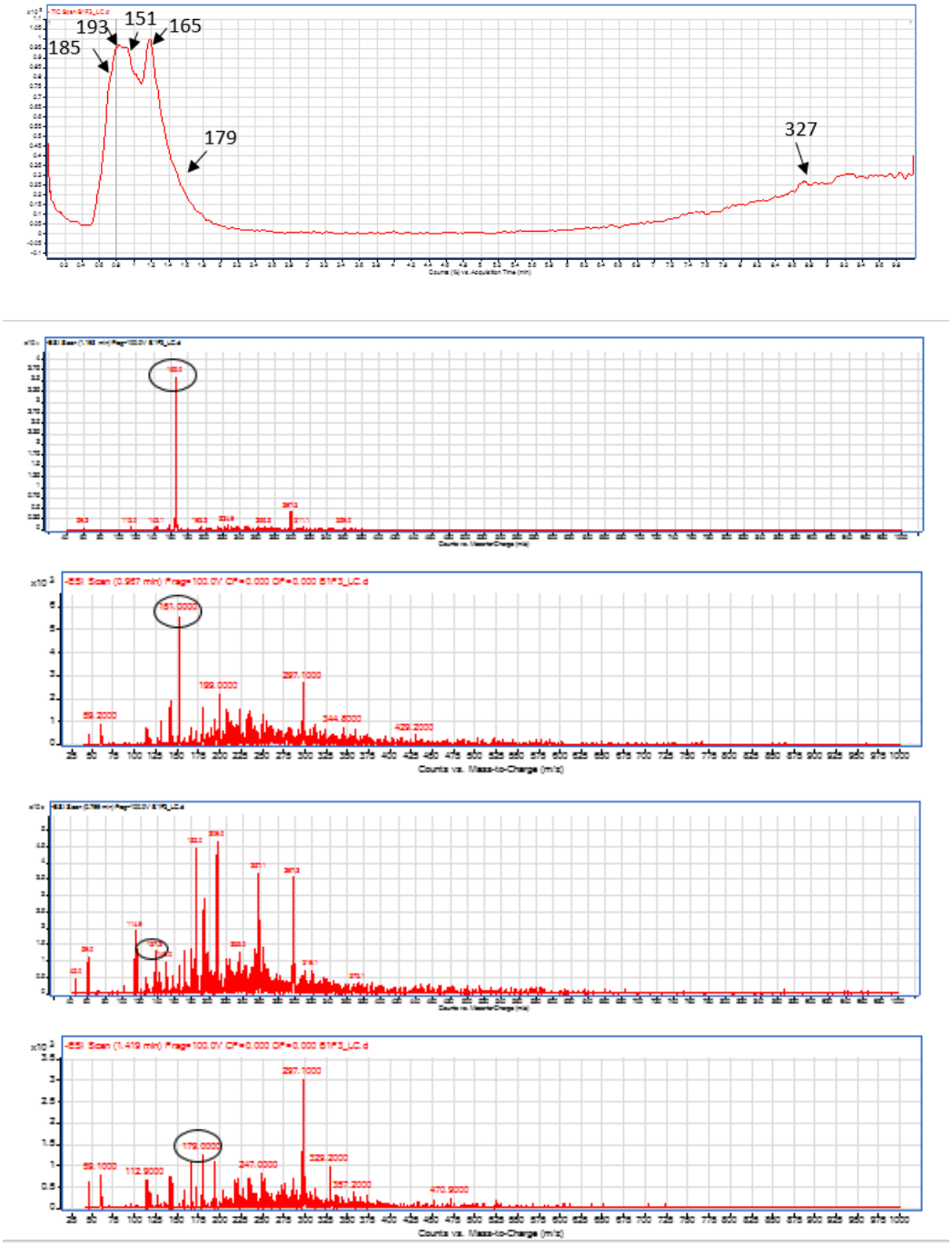


Figure 6.19: TIC from LC-MS of lignin bio-oil (AS.II.E. 3.2.2) and the corresponding spectrum. Showing in black circles, *m/z* 165 (propyl guaiacol), *m/z* 151 (propylbenzene-1,3-diol), *m/z* (propyl guaiacol), *m/z* 179 (guaiacol acetone) respectively, including other masses. Some of the masses are indicated with arrows in the region they are found in the resolved peaks.

Major molecular mass in *Figure 6.19*									
114	193	151	165	185	199	209	247	297	

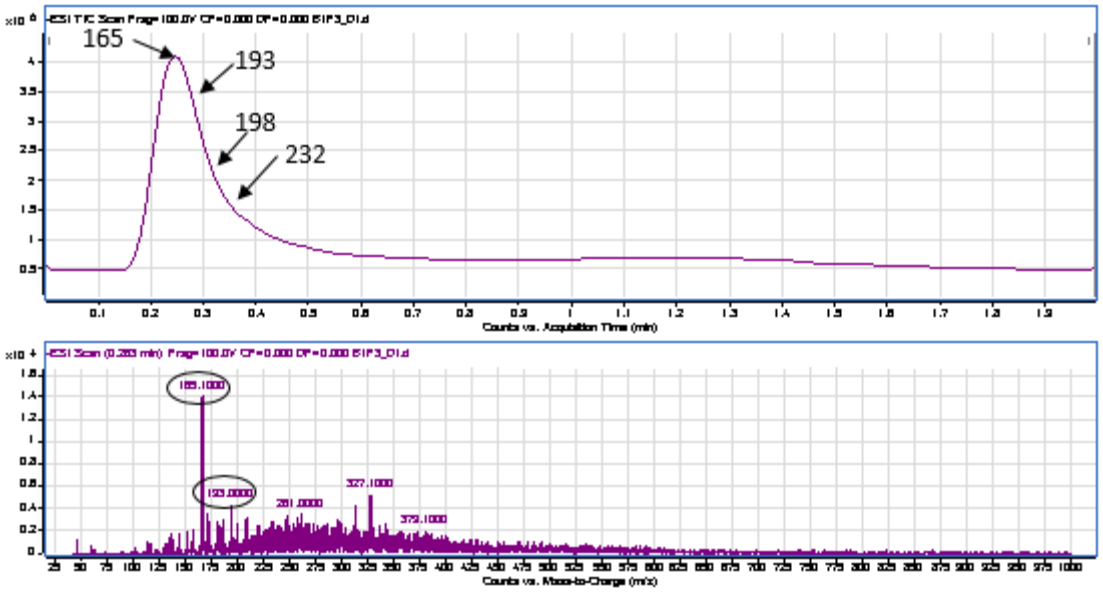


Figure 6.20: TIC from DI-ESI of lignin bio-oil (AS.II.E.3.2) and the corresponding spectrum. Showing *m/z* 165 (propyl guaiacol) and other masses in black. Some of the masses are indicated with arrows in the region they are found in the resolved peak.

Major molecular mass in *Figure 6.20*					
165	193	198	232	247	327

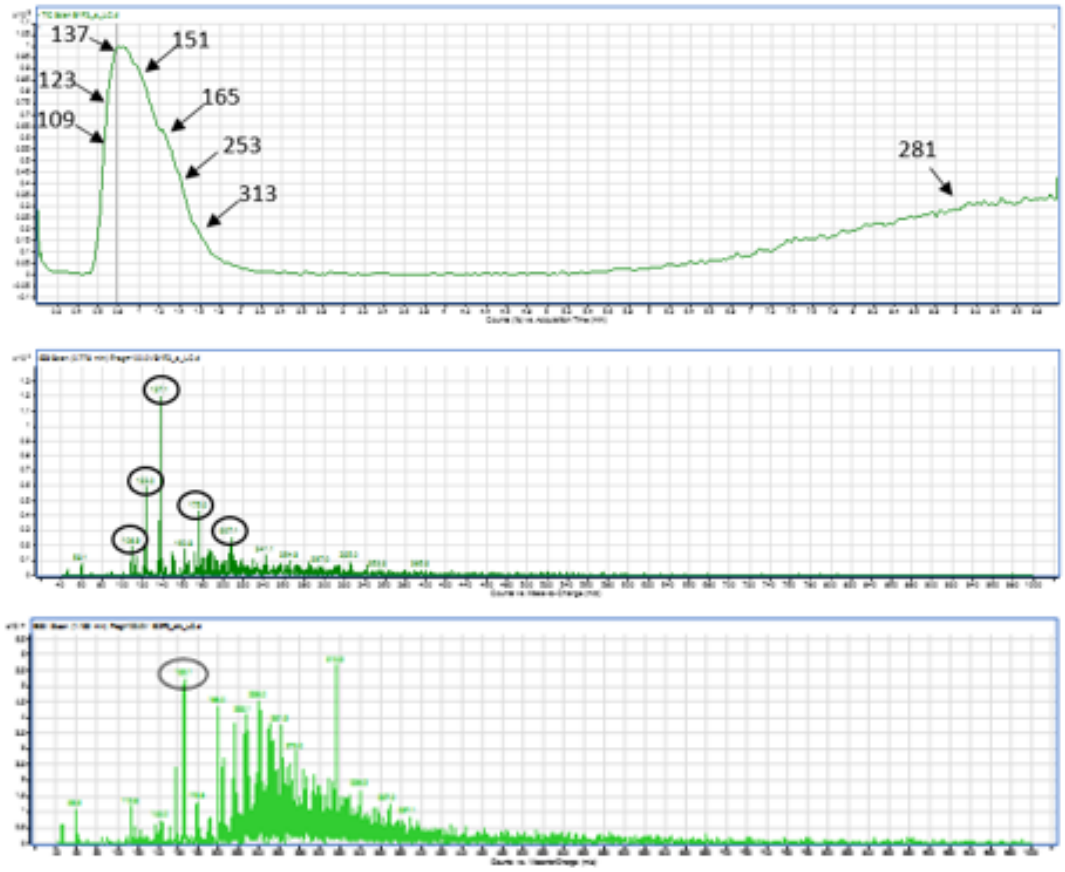


Figure 6.21: TIC from LC-MS of lignin bio-oil (AS.II.3.2) and the corresponding spectrum. Showing *m/z* 109 (DHB or catecol), *m/z* 123 (methyl catecol or guaiacol), *m/z* 137 (ethyl catecol), *m/z* 151 (propylbenzene-1,3-diol) respectively from left to right in black circles in the first spectra and *m/z* 165 (propyl guaiacol) circled in black in the second spectrum. Some of the masses are indicated with arrows in the region they are found in the resolved peaks.

Major molecular mass in *Figure 6.21*									
109	123	137	151	160	165	175	207	241	264

7.0 DISSCUSION

7.1 Method discussion

The LC-ESI-MS method was develop using a reverse phase column with a gradient solvent program (Table 5.2). The instrumental parameters (Table 5.3) were optimised to get adequate conditions to separate and detect the target compounds. The reproducibility of the method was evaluated using the standard compound mixtures (5.1.5) by repeated (intra) injection, the method produced reproducible retention times on repeated (up to 5 times) injection with an acceptable variation of ±0.5mins.

In selecting the mobile phase compositions, a mixture traingle (Figure 5.1) was employed. This design was used to determine the region of possible separation among the three choice solvents which included methanol, acetonitrile and water. After evaluating the resulting chromatograms, water:actonitrile (Figure 5.4) was found adequate for the method development, 0.1% acetic acid was added to increase the resolution and the compositons were optimised to get the most adequate gradient program (Table 5.2) for the method.

However, a likely challenge that would rule out a solvent composition involving methanol even if it was found to be adequate in the separation. The analytes have readily reactive functional groups owing to the high polarity. It was inferred that the functional groups of the target compounds would make methanol a bad candidate to be employed in the gradient program. The -OH, -COOH groups are susceptible to form methyl esters (or ethers) which would give produce inaccurate peaks in the chromatogram [40]. Nevertheless, this inference has not been comfirmed by experiments in the present work.

The optimisation program reported in this work exclusively uses water and acetonitrile composition as the feasible solvents that gave a promising possible separation, see Figure 5.4. It is pertinent to note that solvent strength is the most effective powerful tool available to alter resolution between analytes in LC, not neglecting the role of temperature as opposed to GC where temperature maybe the most important factor in optimising separation and resolution of compounds.

7.1.1 Reverse phase against normal phase

The chemistry of some of the target compounds of interest favours the use of a normal phase chromatography in its separation. In the present work this was taken into consideration, unfortunately this option was not feasible owing to use of an electrospray (ESI) mass spectrometry interface. The ESI system in the lab is not compatible with a normal phase LC system.

Additionally, octadecylsilane-bonded silica (ODS) reverse phase columns are the most extensively used columns for the liquid chromatography coupled to a quadrupole or ion trap mass spectrometry (LC-MS) analyses of complex mixtures. To achieve suitable retention of polar compounds in such column, ion- pairing reagents are often added to the mobile phase in reversed-phase chromatography. However, ion-pairing reagents suppress the ionization of analytes, particularly during electrospray ionization (ESI), due to competitive ionization of the analytes and the reagents, and are therefore not suitable in this context.

In ion-exchange chromatography, and normal-phase chromatography both sufficiently retain polar compounds [10], though, normal phase columns are mostly difficult to apply in LC-ESI-MS analysis due to the use of non-polar mobile phase and polar compounds could be lost to the packing materials. Moreover, the use of a normal phase solvents will affect the ionisation of the analytes, and this is especially a draw back in the case of ESI-MS as used in the present work.

7.1.2 Polarity and ionisation of the analytes of interests.

From the result of the analyses in the present study, positive electrospray ionisation produces some very complex spectra (Figure 5.5). This observation suggests that positive ionisation may not be suitable for targeting non-polar compounds in complex mixtures such as petroleum and bio-oils. In contrary, negative electrospray produces simple spectra containing polar compounds of interests (O, and N- containing compounds). This is understandably since in negative electrospray the aliphatic and closed ring aromatic moieties are not easily ionised. This also proposes the possibilities for further studies in the target analysis and molecular characterisation of the organic compounds in the mixtures.

Furthermore, during the method development, several problems were encountered, such as likely impurities producing unknown peaks, poor retention of the analytes in the column, poor separation and peak overlaps.

In the case of the unknown peaks, after several injections and repeated trials the peaks keep coming up, mostly eluting in acetonitrile around 11-12mins at the near end of

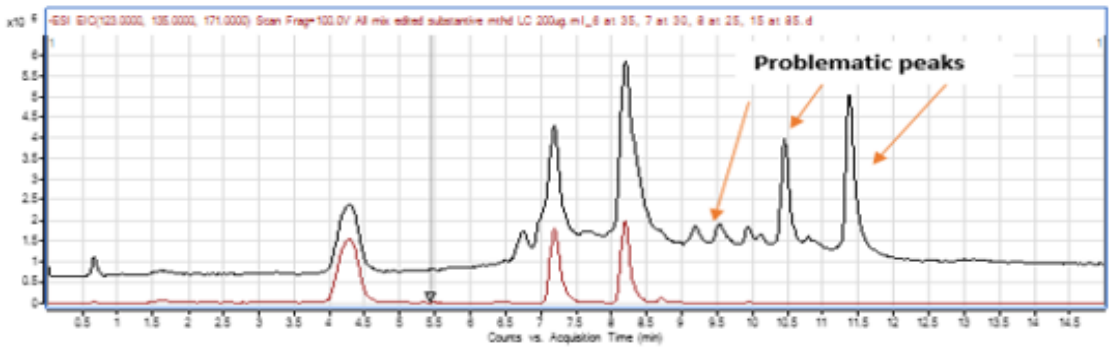


Figure 7.1: TIC from LC-MS showing the well resolved problematic peaks (orange arrows) from the micropipette Eppendorf tips. The EIC in reds below the TIC, shows the separated peaks of guaiacol (*m/z* 123), methyl benzoic acid (*m/z* 135) and naphthoic acid (*m/z* 171) respectively.

the gradient program even when a blank sample was injected into the column, see the chromatogram in Figure 7.1.

This could be related to the sample preparation. Specifically, the first thought was that the masses of 275, 287, 303, 331 etc., were peaks that could likely come from the leaching from the micropipette Eppendorf tips.

The entire standard sample preparation protocols for the standards were revaluated using only glassware and avoiding any material made of plastics. On injection of the new preparation, the unknown peaks were eliminated. The result thus, confirmed that the strange peaks were coming from the leaching of micropipette Eppendorf tips, see Figure 7.2.

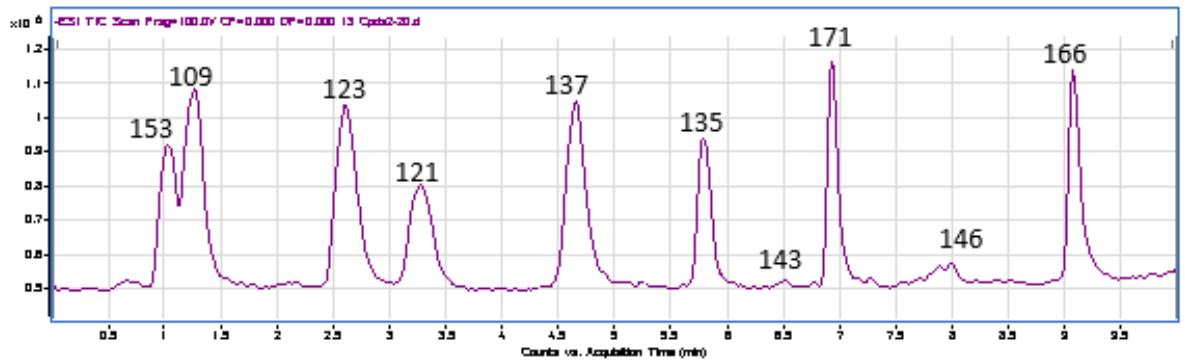


Figure 7.2: TIC from LC-MS of the standards in a mixture, after revaluation of the sample preparation. The *m/z* 153 represents DHBA, 109 represents DHB, 123= methyl catecol, 121= benzoic acid, 137= ethyl catecol, 135= methyl benzoic acid, 143= naphthol, 146= quinolinol, 166= carbazole.

Moreover, the retention of the analytes in the primary column installed on the instrument Zorbax SB-C18, RRT; 2.1×50 mm, 1.8 µm, was poor, however the retention times improved when Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7µm was used. On increasing acetonitrile concentration (plus 0.1% acetic acid) to as high as 60% (Table 5.2) on a Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm; these conditions protonated the analyte and enabled higher MS signal intensity, good separation and resolution.

Also, the analytes peak became well resolved in column 2, Figure 7.3 when the flow rate was increased from 0.3mL/min to 0.5mL/min while keeping other conditions (Table 5.3) constant.

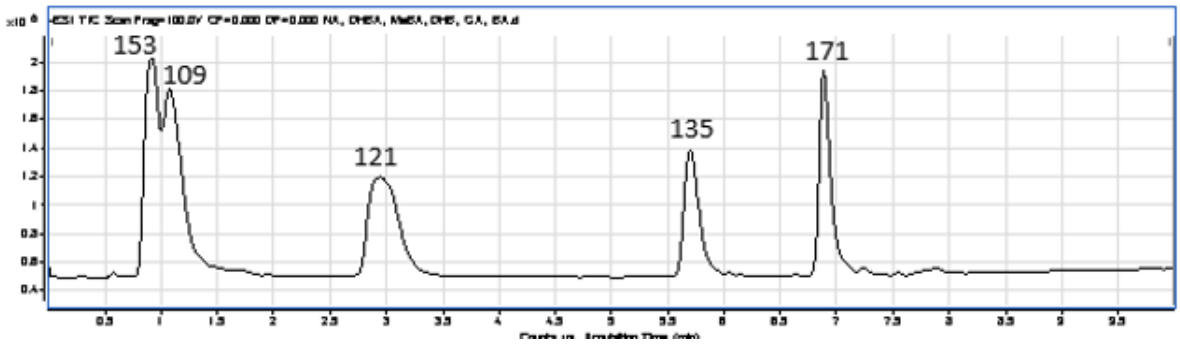


Figure 7.3: TIC from LC-MS showing the resolution of dihydroxybenzoic acid (*m/z* 153), 2,3-dihydroxybenzene (*m/z* 109), benzoic acid (*m/z* 121), methyl benzoic acid (*m/z* 135) and naphthoic acid (*m/z* 171) when the flow rate was increased to 0.5mL/min.

Peak overlaps, and poor retention were circumvented by optimising the gradient program and altering the solvent strengths with several trials. Table 5.2 outlined the most adequate gradient programm for the method.

Nevertheless, this was dependent on the type of column, as column 1 produced optimal peak shapes and fair resolution at flow rate of 0.3mL/min which is well attributed to the very tight packing material with small pore sizes (2.1× 50 mm, 1.8 µm), while column 2 (3.0 × 50 mm, 2.7 µm) was best at flow rate of 0.5mL/min. Injection of 1-2µL was suitable for column 1 while for column 2, 2-3µL injection was found adequate. The packing in column 2 allows the use of low pressure with increased flow rate up to 0.5mL/min of the eluents. The separation efficiency of the column improved throughput and higher resolution in the analytes standards during the method development. The reproducibility (intra) (Figure 5.26) from injection to injection produced consistent results with an acceptable variations between injections. The selectivity mainly for compounds with carboxylic, methoxyl and hydroxyl functional groups were adequate.

In addition, during the course of the method development several optimisation process were evaluated. Some of the tried gradient programs, instrumental parmaters and their corresponding chromatograms can be found in the appendix of this thesis.

7.2 Characterisation of lignin bio-oils and petroleum

Petroleum and lignin derived bio-oils are complex mixtures and their characterisation often poses a great challenge to analytical scientists; particularly, developing a suitable analytical method is undoubtedly tasking.

Previous work in our group by *Audun Kronstad* [41] had attempted to developed DI-ESI-MS method for characterisation of compounds found in lignin derived bio-oils. In his work, he was not able to develop a suitable condition that could ionise the compounds either in a single preparation or mixtures of several compounds which included guaiacol, amino phenol among others. *Kronstad* had used methanol [+0.1% formic acid] as a mobile phase in the system. In his work, *Kronstad* mentioned switching between polarities and variation in needle voltage as well as fragmentor settings. However, he did not report the exact values of the optimisation processes for comprehensive comparisons. Section (7.1) of the present thesis pointed out why the use of methanol could give erroneous peaks and incorrect results due to the types of target analytes in question. In this work, I have established ESI conditions that can ionise the compounds as well as a liquid chromatography method that can separate the standard compounds in the mixtures.

7.2.1 Petroleum

The developed analytical method (5.1), liquid-chromatography hyphenated with Electrospray mass spectrometry (LC-ESI-MS) has been applied to separate different compounds in the fractions from the SPE procedure as well as in whole oils and ESI has been use for the molecular identification of the compounds per their molecular weights. The fractions are assumed to contain most of the polar fractions. As can be seen in the chromatogram (Figure 6.1-6.6) there is no clear separation of the compounds in the real petroleum samples. Some molecular masses analogous to the standard compounds used in the method development were seen in the mass spectral of the fingerprinted crude oil samples. Some peaks corresponding to *m/z* 121 (benzoic acid) and *m/z* 171 (naphthoic acid) are evident, see black circles in Figure 6.2. Going further in, Figure 6.4 *m/z* 143 (naphthol) and *m/z* 171 (naphthoic acid) can be seen in the spectra of the *Heidrun* (biodegraded oil), see also Table 7.1. It can therefore be inferred that the biodegraded oils contain more of naphthene rings than the other oils under study.

Furthermore, microbial alteration of crude oils confers it compounds of higher molecular weights, since the lighter hydrocarbons are removed by the microbial alteration [11].

7.2.2 Bio-oils

In strong contrast to the petroleum samples both in whole and fractionated oils. LC of the lignin derived oils showed more success in separation and identification of the target molecules (Table 7.1) in the samples. The chromatograms of non-fractionated (Figure 6.7-6.10) and fractionated (Figure 6.15-6.21) lignin bio-oils with the corresponding mass spectra showing *m/z* 109 (catecol or dihydroxybenzene), *m/z* 137 (ethyl catecol), *m/z* 143 (naphthol), *m/z* 123 (methyl catecol or guaiacol), *m/z* 151 (propylbenzene-1,3-diol), *m/z* 165 (propyl guaiacol), *m/z* 177 (methoxy eugenol), and *m/z* 179 (guaiacol acetone), see Figure 2.8, shows a success in the identification of molecular masses of interest. Including other masses such as 175, 191,199, 243, 285 etc., these masses could be further explored to identify compounds that produced the spectra in mass spectrometry instrument.

7.3 Effects of fractionation and non-fractionation on the samples

In the case of crude oil, fractionation in general does not have any noticeable effect on the type of polar compounds seen in the mass spectral trace when compared to the fingerprinting approach. The only noticeable difference is in the background noise; the non-fractionated oils have more background spectra when compared to the fractionated oils (see Figure 6.1 and Figure 6.12).

However, in the case of the bio-oils, direct injection of non-fractionated samples produced two peaks (Figure 6.7-6.10) while direct injection of fractionated oils produced single peaks (Figure 6.17 and Figure 6.20). Table 7.1 below summarises the compounds identified in the mass spectrometry instrument in both fingerprinted and fractionated samples. It can also be seen from the various chromatograms that fractionation did not have any effect on the ability of the method to separate the various compounds, except in Figure 6.19 that showed some promising separation. In Figure 6.11, where fingerprinting approach was used, there was some unique semblance of separation of some compounds. Some of the peaks in Figure 6.11 corresponds to retention times from the standards. For instance, *m/z* 109 (catecol or DHB) eluted at

approximately 1.2 mins, and two different peaks of *m/z* 123 eluted at 2.2 and 2.4 mins, which may correspond to methyl catecol and guaiacol see Table 5.4.

Table 7.1: List of identified compounds found in the bio-oil and petroleum samples.

Lignin Bio-oils, (m/z)	**Probable compounds**
123	Guaiacol or methyl catecol
109	Catecol or dihydroxybenzene
137	Ethyl catecol
198	Guaiacol glyceryl ether
151	Propylbenzene-1,3-diol
177	Methoxy eugenol
179	Guaiacol acetone
165	Propyl guaiacol

Petroleum, (m/z)	**Probable compounds**
143	Naphthol
171	Naphthoic acid
121	Benzoic acid

Additionally, the mass spectra of direct injection of bio-oils are dominated by spacing of 28 Da (CH_2-CH_2) and 14 Da (CH_2). Similar patterns (14 Da) has been previously reported [19, 20, 42]. This suggests different homologous series with basic chemistry and varying number of CH_2-CH_2 and CH_2 units.

7.4 LC-ESI-MS or DI-ESI

7.4.1 Petroleum samples

From the results of the present work, running the petroleum oils through the column have no significant impact. The retention times in the standard compounds were not replicated throughout the LC-MS of the real samples, as there was no visible separation. The big question is why there was no separations in the column as found in the standard compounds (Figure 5.25). One of the reasons can be deduced from the complexity of petroleum samples. The standards are pure compounds while the real samples are complex mixtures and such complexity can affect the separation efficiency of the analytes. Other reasons may include matrix effect, less efficiency of the column due to overload, blockage of the column active sites which will ultimately inhibit interaction of the polar compounds with the column packing materials, forcing such compounds to be washed off without retention. These reasons as presumed may have affected the separation efficiency of the analytes and the subsequent non-uniformities of the retention times as found in the standard mixtures.

7.4.2 Bio-oil samples

The developed LC method showed more success in the separation of some of the lignin derived bio-oil samples. For instance, most of the peaks in Figure 6.11 are clearly separated and resolved, which did not reflect in the petroleum samples.

Furthermore, most separation scientists during an analytical method development encounters methods that fail to separate important components of a mixture. However, in this case it is somewhat strange to the idea of chromatography as the analogues used in the method development are expected to replicate the same behaviour in the real samples. This proposes more study on the LC part of the method to find a possible answer to the reflection.

Thus, from the observation, the author of the present work suggests that anyone who intends to use the present method for characterisation of the polar compounds in the oils should use the direct injection into the ESI, without separation on a chromatographic column, especially for petroleum samples.

8.0 Conclusion

A liquid chromatography electrospray ionisation mass spectrometry method for the separation and profiling of organic compounds in crude oil and lignin derived bio-oil mixtures has been developed. The analytical method was developed in a reverse phase chromatography with gradient elution program using water and acetonitrile plus 0.1% acetic acid. The method was developed using standard compounds which include, naphthoic acid, naphthol, methyl benzoic acid, guaiacol, guaiacol glyceryl ether, catecol, dihydroxybenzene, dihydroxybenzoic acid, methyl catecol, ethyl catecol, quinolinol, NH_2-benzamide, and carbazole. The separation is reproducible over repeated injection of the standard compounds with an acceptable variation in the retention times.

A solid phase extraction protocol using a normal phase cyano-bonded stationary phase was modified to fractionate the petroleum and bio-oils into polar and non-polar components. Hexane/dichloromethane was used to elute the non-polar and hydrocarbon components of the bio-oil and petroleum respectively, while acetonitrile and methanol was used to elute the polar fractions which were submitted to the reverse phase column for separation and profiling.

The application of the DI-ESI-MS and LC-ESI-MS method both in direct fingerprinting and polar fractions of the both oils showed that molecular masses of interest were detected. These include, *m/z* 121 (benzoic acid), *m/z* 143 (naphthol) and *m/z* 171 (naphthoic acid) for crude oils. In bio-oils, m/z 109 (catecol or dihydroxybenzene), *m/z* 137 (ethyl catecol), *m/z* 143 (naphthol), *m/z* 123 (methyl catecol or guaiacol), *m/z* 151 (propylbenzene-1,3-diol), *m/z* 165 (propyl guaiacol), *m/z* 177 (methoxy eugenol), m/z 179 (guaiacol acetone), and 175, 191, 193, 199, 243, 285 etc., were detected.

Notwithstanding, structural and data analyses is needed to establish the certainty of the detected compounds and their chemistry. As in mass spectrometry, several compounds with same molecular weight maybe assigned same *m/z* (even if they may have different retention times) especially in a low-resolution mass spectrometry instrument as used in this study.

9.0 Proposals for further work

Using software to analyse the spectra for further study and characterisation.

The research proposes the potential of applying multivariate data analyses to explore the data of the spectra from ESI-MS and the LC-MS results.

Exploring the data to get more information on the plethora of other masses found in the spectra.

The method can further be tested in a high-resolution mass spectrometry for certainty of the ions produced and corresponding spectra.

REFERENCES

[1] J. G. Speight, *The Chemistry and Technology of Petroleum*, Second, revised and expanded ed. Marcel Dekker, Inc, 1991, pp. 328-329.

[2] J. G. Speight, *Petroleum Chemistry and Refining*. Washington, DC: Taylor & Francis, 1998, pp. 9-16.

[3] J. Wertz and O. Bedue, *Lignocellulosic Biorefineries*. EPFL Press, 2013, pp. 240-289.

[4] J. Sadhukhan, K. S. Ng, and E. M. Hernandez, *Biorefineries and Chemical Processes: Design, Integration and Sustainability Analysis*, First ed. The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, United Kingdom: John Wiley & Sons, Ltd, 2014.

[5] D. M. Mousdale, *Biofuels, Biotechnology, Chemistry and Sustainble Development*. New York: CRC Press, 2008.

[6] H. Schobert, *Chemistry of Fossil Fuels and Biofuels*. Cambridge University Press, 2013, pp. 181-185.

[7] O. C. Mullins, E. Y. Sheu, A. Hammami, and A. G. Marshall, *Asphaltenes, Heavy Oils, and Petroleomics*. New York, USA: Springer, 2007, pp. 1-59, 617-622.

[8] K. Erstad, S. Hoiland, P. Fotland, and T. Barth, "Influence of Petroleum Acids on Gas Hydrate Wettability," *Energy & Fuels*, Article vol. 23, pp. 2213-2219, Mar-Apr 2009.

[9] R. R. Colwell, J. D. Walker, and J. J. Cooney, "Ecological aspects of microbial degradation of petroleum in the marine

environment," *Critical Reviews in Microbiology*, vol. 5, no. 4, pp. 423–445, 1977.

[10] A. E. Borgund, K. Erstad, and T. Barth, "Normal phase high performance liquid chromatography for fractionation of organic acid mixtures extracted from crude oils.," *J. Chromatogr A*, vol. 1149, pp. 189-196, 2007.

[11] T. Barth, S. Høiland, P. Fotland, K. M. Askvik, B. S. Pedersen, and A. E. Borgund, "Acidic compounds in biodegraded petroleum," *Organic Geochemistry*, vol. 35 pp. 1513–1525, 2004.

[12] W. Ulrici, H. J. Rehm, and G. Reed, "Contaminant soil areas, different countries and contaminant monitoring of contaminants," *Environmental Process II. Soil Decontamination Biotechnology*, vol. 11, pp. 5-42, 2000.

[13] J. J. Cooney, S. A. Silver, and E. A. Beck, " Factors influencing hydrocarbon degradation in three freshwater lakes," *Microbial Ecology,*, vol. 11, no. 2, pp. 127-137, 1985.

[14] P. Rikka, "Spectrometric identification of naphthenic acids isolated from crude oil," Master of Science thesis, Texas State University-San Marcos, 2007.

[15] M. J. Wilde and S. J. Rowland, "Structural Identification of Petroleum Acids by Conversion to Hydrocarbons and Multidimensional Gas Chromatography-Mass Spectrometry," *Anal. Chem.*, vol. 87, p. 8457−8465, 2015.

[16] F. A. Goncalves, H.A. Ruiz, E.S. dos Santos, a. J.A Teixeira, and G. R. d. Macedo., "Bioethanol production by Saccharomyces cerevisiae, Pichia stipitis and Zymomonas mobilis

from delignified coconut fibre mature and lignin extraction according to biorefinery concept," *Renewable Energy,* vol. 94, pp. 353-365, Aug 2016.

[17] B. Holmelid, M. Kleinert, and T. Barth, "Reactivity and reaction pathways in thermochemical treatment of selected lignin-like model compounds under hydrogen rich conditions," *J. Anal. Appl. Pyrolysis,* vol. 98, pp. 37–44, 2012.

[18] J. R. Gasson, M. Kleinert, I. Eide, and T. Barth, "Lignin Solvolysis: Upscaling of the Lignin-to-Liquid conversion process towards technical applicability

" presented at the 18th European Biomass Conference & Exhibition, At Lyon, France, 2010.

[19] G. Gellerstedt, J. Li, I. Eide, M. Kleinert, and T. Barth, "Chemical Structures Present in Biofuel Obtained from Lignin," *Energy & Fuels 2008* vol. 22, pp. 4240–4244, 2008.

[20] J. R. Gasson, M. Kleinert, I. Eide, and T. Barth, "Developing solvolytic conversion of lignin to liquid (LtL) fuel components: Optimisation of quality and process factors," *Cellulose Chemistry and Technology,* vol. 45, no. 1, pp. 3-12, 2011.

[21] C. Løhre, T. Barth, and M. Kleinert, "The effect of solvent and input material pretreatment on product yield and composition of bio-oils from lignin solvolysis," *J. Anal. Appl. Pyrolysis,* vol. 119, pp. 208-216, 2016.

[22] B. Shrestha, M. Oregui, C. Løhre, and T. Barth, "The effects of catalysts on the reaction temperature and duration requirements in solvolytic lignin conversion," presented at the 19th International Symposium on Analytical and Applied Pyrolysis, At Linz, Austria, 2013.

[23] J. M. Miller, *Chromatography Concepts and contrasts*. Hoboken New Jersey: John Wiley & Sons, Inc., 2005, p. 490.

[24] R. O. Abia, "Development and validation of a novel method for serotonin and 5-hydroxyindole-acetic acid determination in plasma using liquidchromatography tandem mass spectrometry," Master Thesis, Department of Chemistry, University of Cadiz, and University of Bergen, Bergen, Norway, 2014.

[25] J. E. Carlson, J. R. Gasson, T. Barth, and I. Eide, "Extracting homologous series from mass spectrometry data by projection on predefined vectors," *Chememotrics and Intelligent laboratory systems,* vol. 114, pp. 36-43, 2012.

[26] W. Jie, X. F. Cao, L. W. Chai, J. Q. Liao, Y. Huang, and X. Y. Tang, "Quantification and characterization of naphthenic acids in soils from oil exploration areas in China by GC/MS," *Analytical Methods,* vol. 7, no. 5, pp. 2149-2154, 2015.

[27] R. P. Rodgers, C. A. Hughey, C. L. Hendrickson, and A. G. Marshall, "Advanced characterization of petroleum crude and products by high field Fourier Transform ion cyclotron resonance mass spectrometry " *Fuel Chemistry Division Preprints,* vol. 47, no. 2, p. 636, 2002.

[28] J. Viidanoja, "Analysis of phospholipids in bio-oils and fats by hydrophilic interaction liquid chromatography-tandem mass spectrometry," *J. Chromatogr B-Analyt Technol Biomed Life Sci.,* vol. 1001, pp. 140-149, Sep 2015.

[29] I. Eide and K. Zahlsen, "A Novel Method for Chemical Fingerprinting of Oil and Petroleum Products Based on Electrospray Mass Spectrometry and Chemometrics," *Energy & Fuels , ,* vol. 19, pp. 964-967, 2005.

[30] A. L. Tomren, T. Barth, and K. Folgerø, "Multivariate Analysis of Crude Oil Composition and Fluid Properties Used in Multiphase Flow Metering (MFM)," *Energy Fuels* vol. 26, pp. 5679-5688, 2012.

[31] E. D. Hoffmann and V. Stroobant, *Mass Spetcromery: Princples and Applications*. John Willey & Sons, Ltd, 2007, pp. 15-80.

[32] R. M. Smith, *Understanding Mass spectra*. Hoboken New Jersey: John Willey & Sons, Inc.,, 2004, pp. 1-52.

[33] C. S. H. C.W.K Lam, M.H.M Chan, R.C.K Cheung, L.K Law, L.C.W Lit, K.F Ng, M.W.M Suen and H.L Tai, "Electrospray Ionisation Mass Spectrometry: Principles and Clinical Applications," *Clin Biochem Rev,* vol. 24, pp. 3-12, 2003.

[34] N. Fang, S. Yu, and R. L. Prior, "LC/MS/MS Characterization of Phenolic Constituents in Dried Plums," *J. Agric. Food Chem. 2002, , ,* vol. 50, no. 3579, p. 3579−3585 2002.

[35] M. Kallenbach, I. T. Baldwin, and G. Bonaventure, "A rapid and sensitive method for the simultaneous analysisof aliphatic and polar molecules containing free carboxyl groups in plant extracts by LC-MS/MS," *Plant Methods,* vol. 5, no. 17 pp. 5-17

2009.

[36] J. Št'ávová, J. Beránek1, E. P. Nelson, B. A. Diep, and A. Kubátová, "Limits of detections for the determination of mono- and dicarboxylic acids using gas and liquid chromatographic methods coupled with mass spectrometry," *J. Chromatogr B Analyt Technol Biomed Life Sci.,* vol. 879, no. (17-18), pp. 1429–1438., 2011.

[37] C. E. Rosta, "Analysis of solvent dyes in refined petroleum products by electrospray ionization mass spectrometry," *Fuel* vol. 89, pp. 997–1005, 2010.

[38] J. H. Christensen, "Chemometrics as a tool to analyse complex chemical mixtures

– Environmental forensics and fate of oil spills," Doctoral thesis, Department of Environmental Chemistry and Microbiology

Roskilde University, Department of Life Sciences and Chemistry and NERI, National Environmental

Research Institute, Denmark, http:/afhandlinger.dmu.dks, 2005.

[39] A. Technologies. (Accessed on February 20, 2017). *LC Columns Deliver Results for Complex Analytical Challenges*. Available: http://www.agilent.com/en-us/products/liquid-chromatography/lc-columns#0.

[40] L. ChromAcademy, "Theory of HPLC Reverse Phase Chromatography," Accessed March 15, 2017, Available: http://www.chromacademy.com/lms/sco5/Theory_Of_HPLC_Reverse_Phase_Chromatography.pdf.

[41] A. Kronstad, "A study of catalytica activity of sulphate H-Ni-Mo catalyt on ZrO2 support in the LtL-process, and development of ESI-MS method for bio-oils," Master, Chemistry, University of Bergen, Bergen, 2015.

[42] I. Eide and G. Neverdal, "Fingerprinting Bio-Oils from lignocellulose and comparison with Fossil Fuels," *Energy & Fuels ,,* vol. 28, pp. 2617-2623, 2014.

Appendix

Optimizing gradient time

Time	A [H_2O] %	B [ACN] %
0	85	15
5	70	30
8	50	50
12	30	70
15	85	15

PARAMETERS:

LC

FR= 0.25mL/min

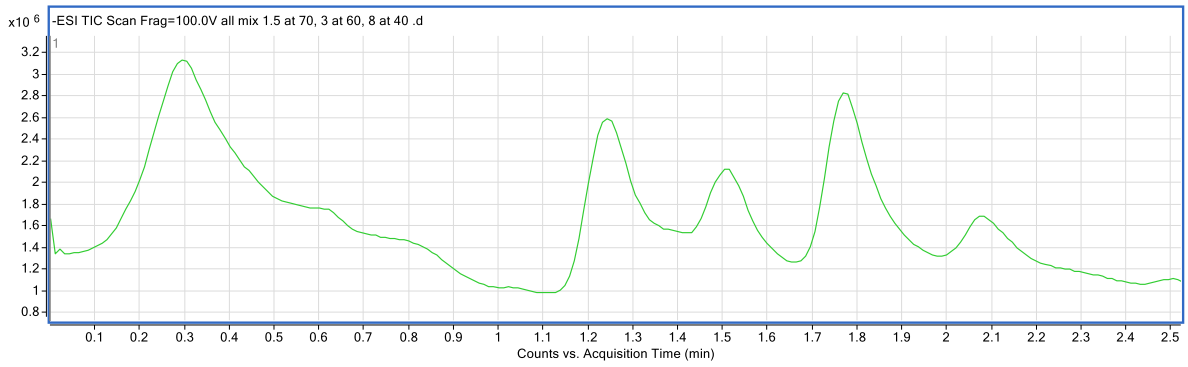
Injection volume: 1µL, Column temperature not controlled but ranges from 20-30°C

MS

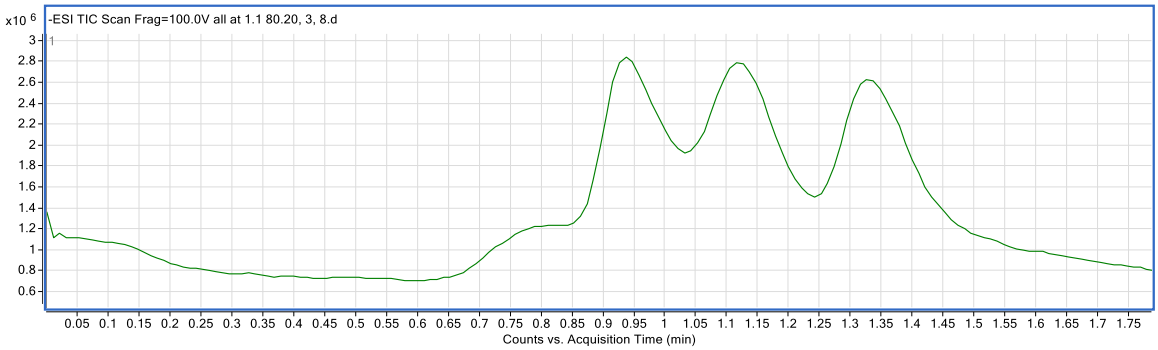
Fragmentor: 100, Scan cycle/sec: 600, Capillary voltage 4000V, Mass range: 40-1000 m/z, Gas flow 8L/min and

Nebulizer: 15 psi

Time	A [H_2O] %	B [ACN] %
0	85	15
2	70	30
8	50	50
12	30	70
15	85	15

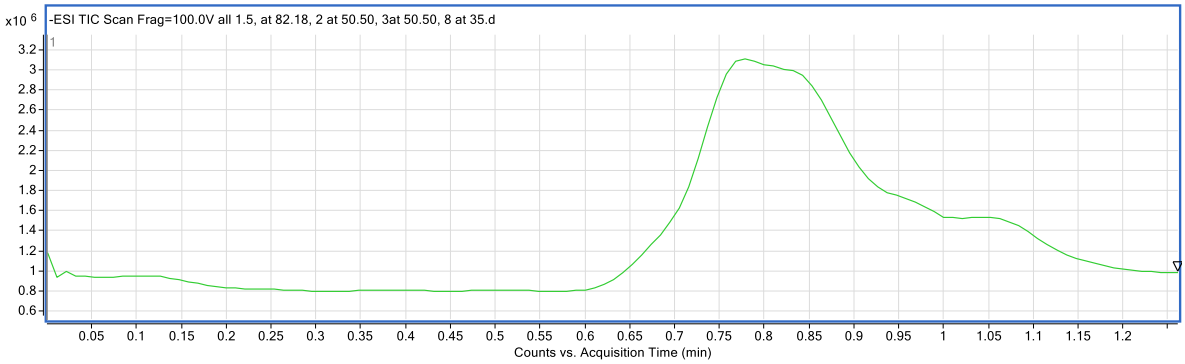


Time	A [H_2O] %	B [ACN] %
0	85	15
1	70	30
3	50	50
8	30	70
15	85	15

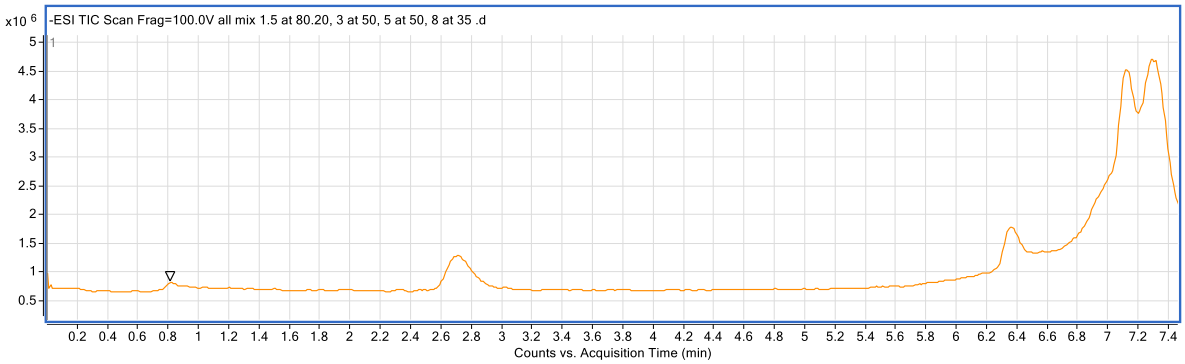


Optimizing gradient flow composition

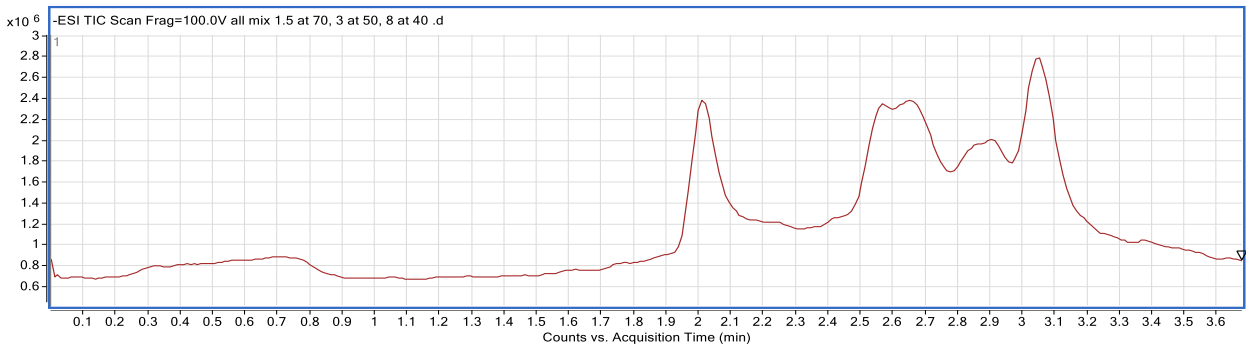
Time	A [H_2O] %	B [ACN]%
0	85	15
1.5	82	18
2	50	50
3	50	50
8	35	65
15	85	15



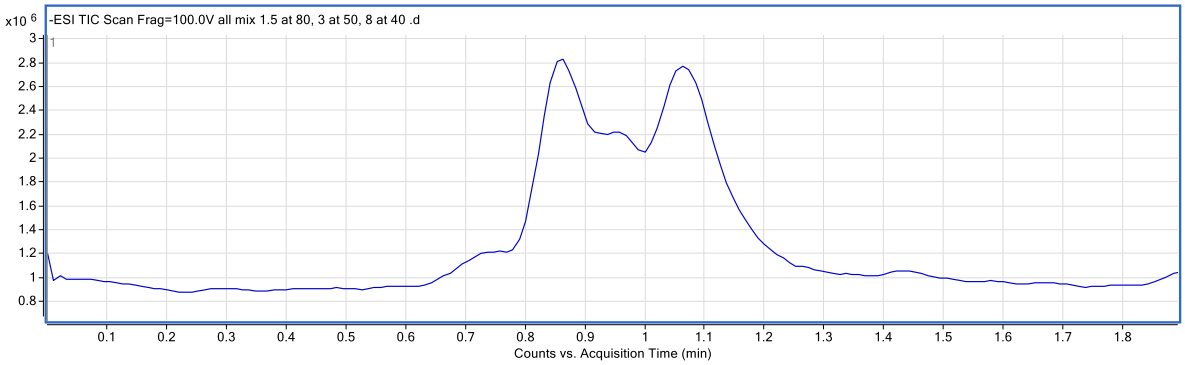
Time	A [H_2O] %	B [ACN] %
0	85	15
1.5	80	20
3	50	50
5	50	50
8	35	65
15	85	15



Time	A [H_2O] %	B [ACN] %
0	85	15
1.5	70	30
3	50	50
8	35	65
15	85	15

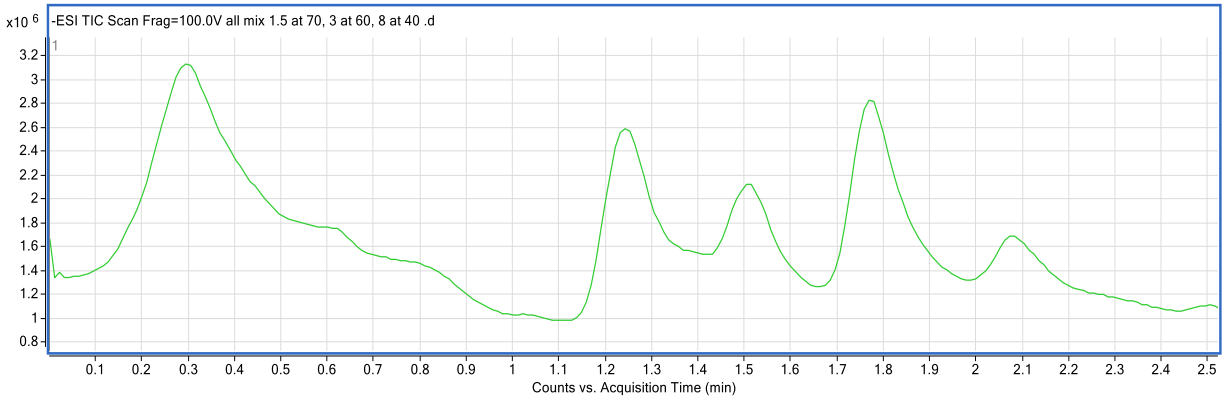


Time	A [H_2O] %	B [ACN] %
0	85	15
1.5	80	20
3	50	50
8	30	70
15	85	15

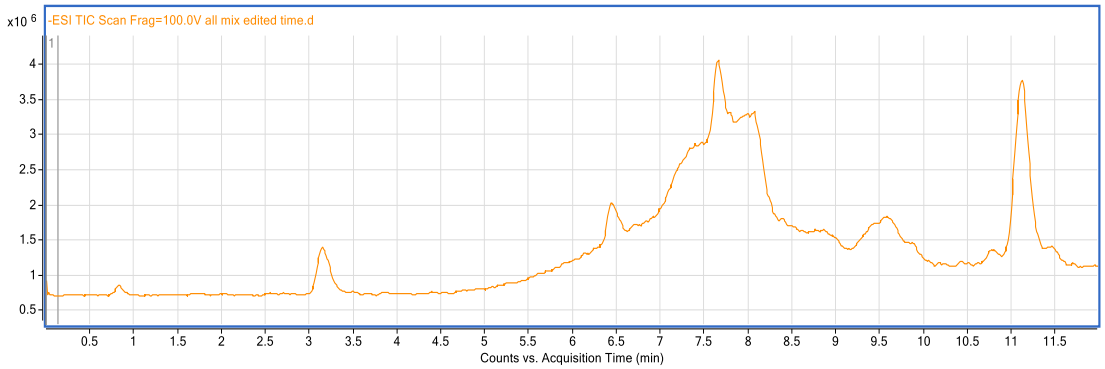


Flow rate (FR) = 0.2 mL/min

Time	A [H_2O] %	B [ACN] %
0	85	15
1.5	70	30
3	60	40
8	40	60
15	85	15

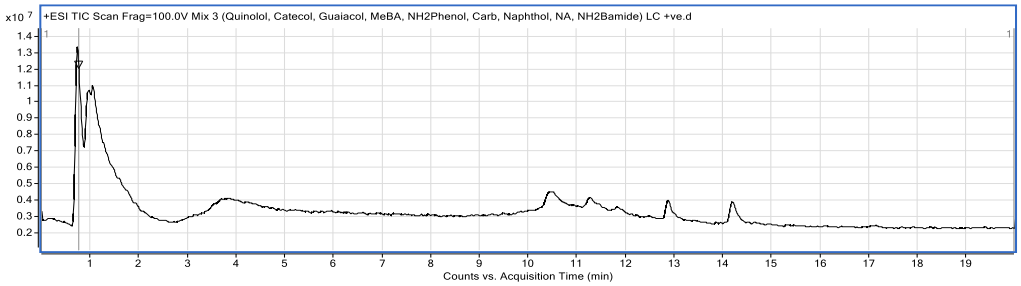


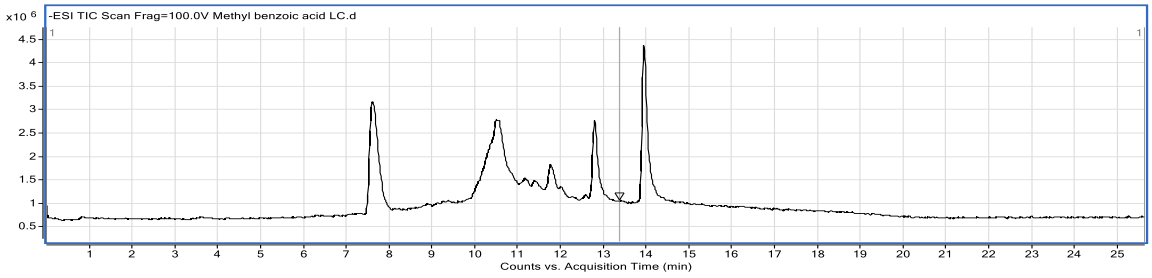
Time	A [H_2O] %	B [ACN] %
0	85	15
2	70	30
3	60	40
8	40	60
15	85	15



Temperature at 40°C
FR = 0.2mL/min

Time	A [H_2O] %	B [ACN] %
0	85	15
2	70	30
3	60	40
8	40	60
15	85	15



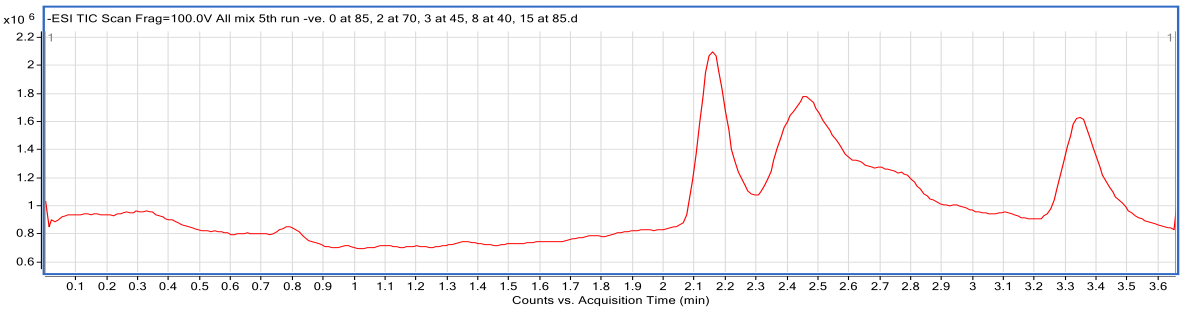


Impurities from eppendorf tips

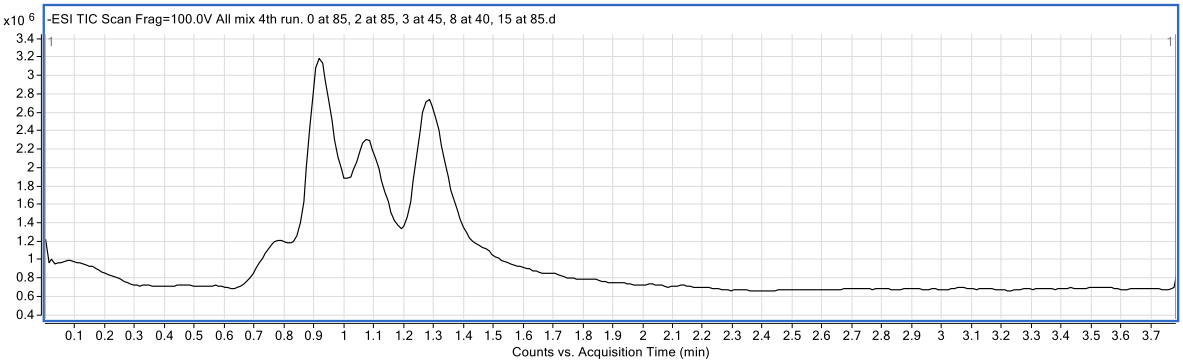
Temperature and gradient flow optimization

Temperature at 50°C
FR = 0.250mL/min

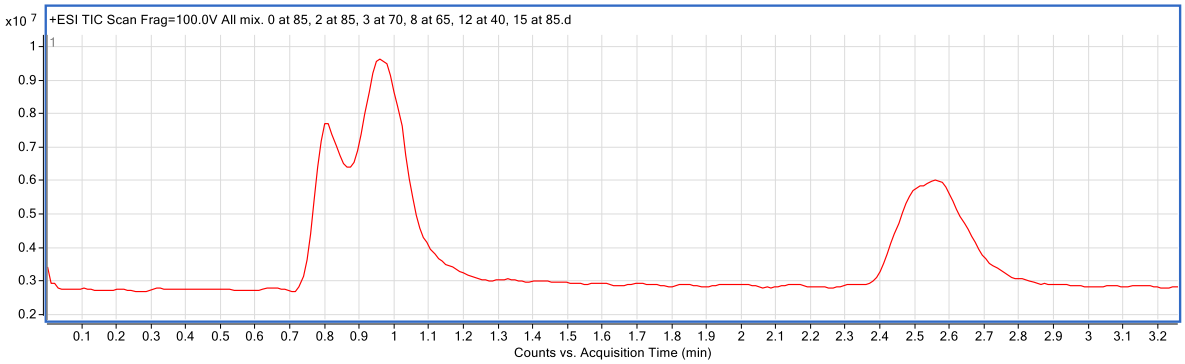
Time	A [H_2O] %	B [ACN] %
0	85	15
2	70	30
3	65	35
8	40	70
15	85	15



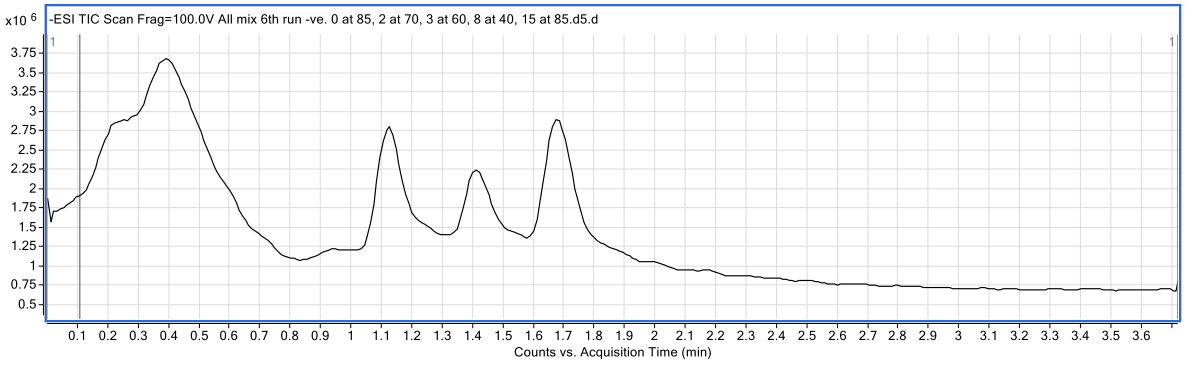
Time	A [H_2O] %	B [ACN] %
0	85	15
2	85	15
3	70	30
8	65	35
12	40	70
15	85	15



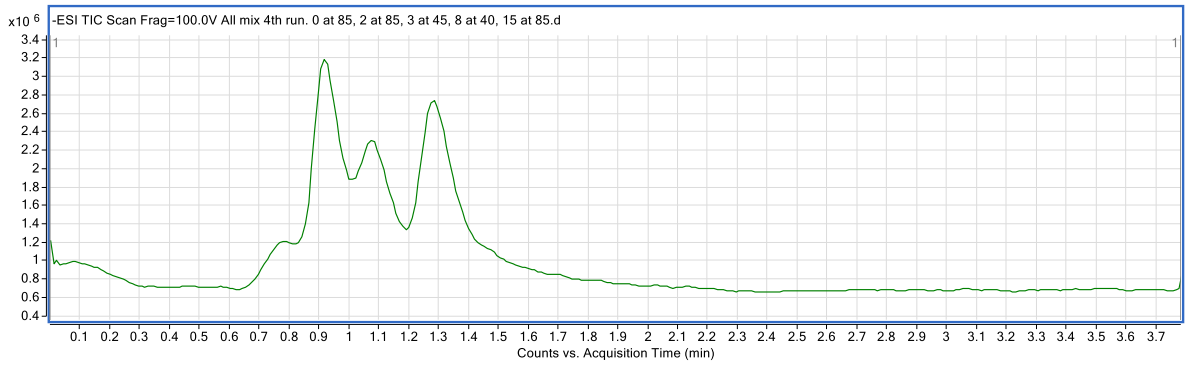
Time	A [H_2O] %	B [ACN] %
0	85	15
5	85	15
8	50	50
12	40	70
15	85	15



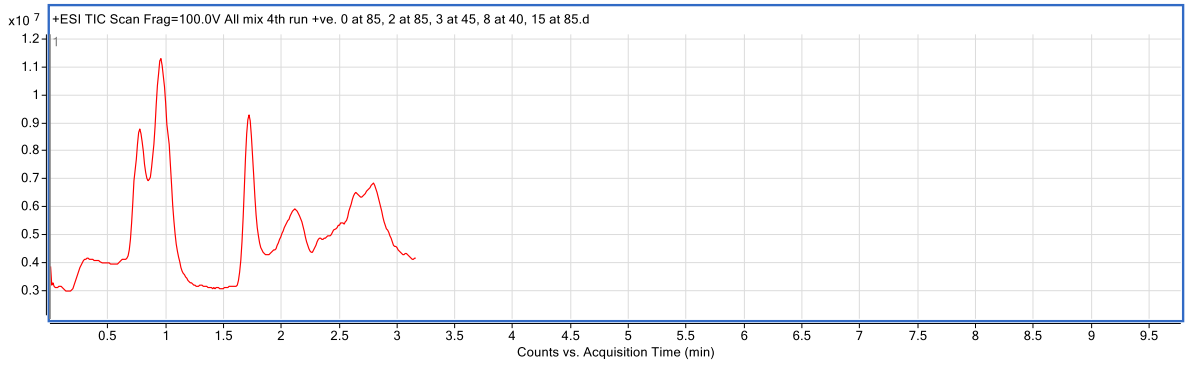
Time	A [H_2O] %	B [ACN] %
0	85	15
2	85	15
3	50	50
8	40	70
15	85	15



Time	A [H_2O] %	B [ACN] %
0	85	15
2	85	15
3	45	55
8	40	60
15	85	15



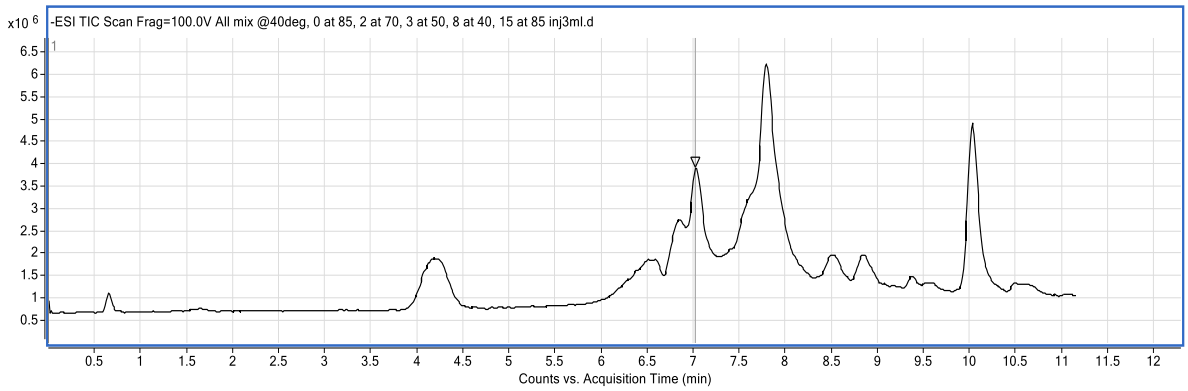
Time	A [H_2O] %	B [ACN] %
0	85	15
2	85	15
3	45	55
8	40	60
15	85	15



NEW COLUMN

Temperature at 40°C
FR = 0.500mL/min

Time	A [H_2O] %	B [ACN] %
0	85	15
2	70	30
3	50	55
8	40	60
15	85	15

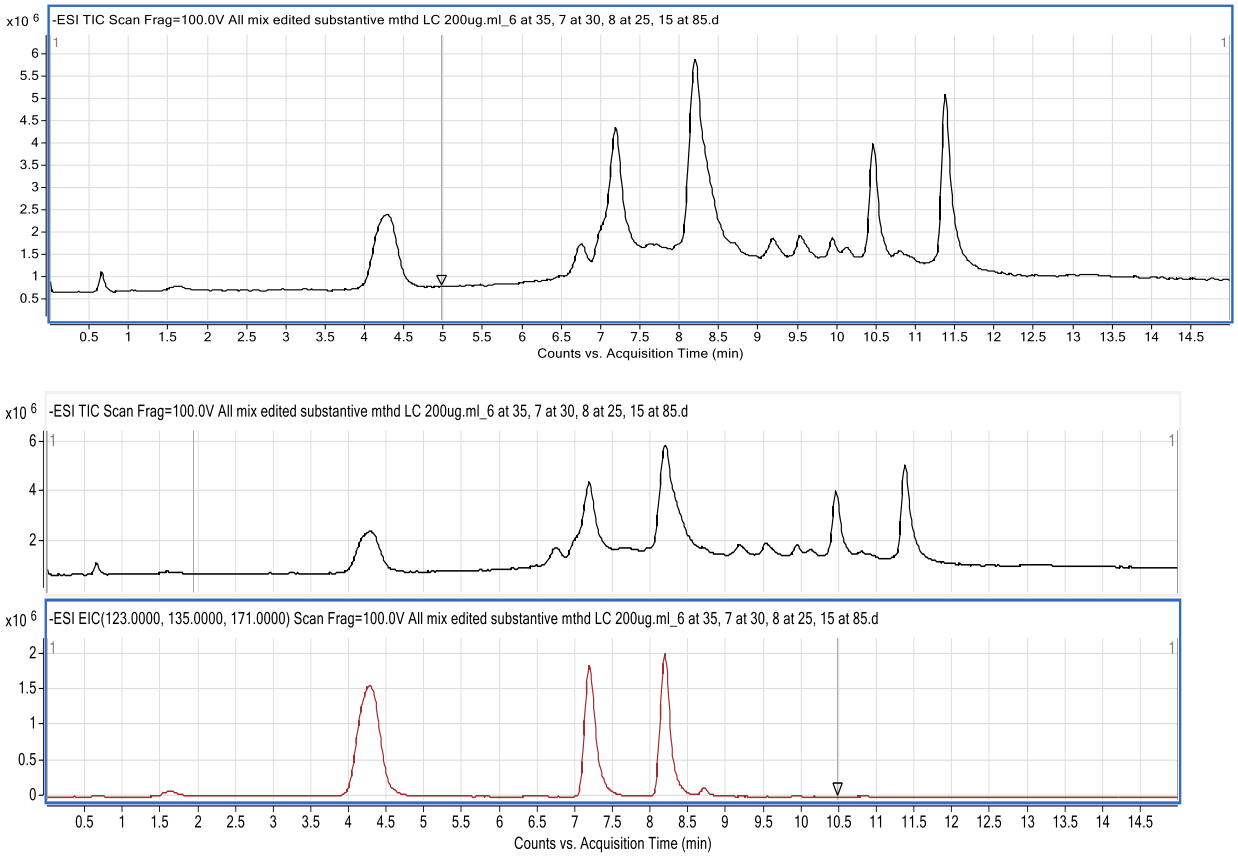


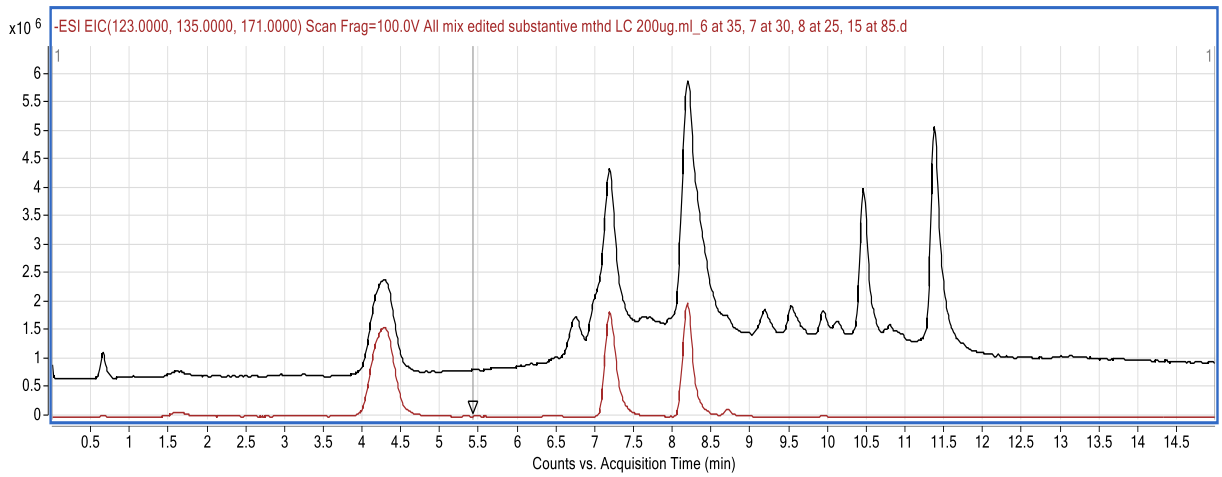
COMMENT: Best separation and good retention times

Time	A [H_2O] %	B [ACN] %
0	85	15
2	70	30
3	60	40
4	55	45
5	50	50
6	45	55
7	35	65 isocratic
8	35	65
15	85	15

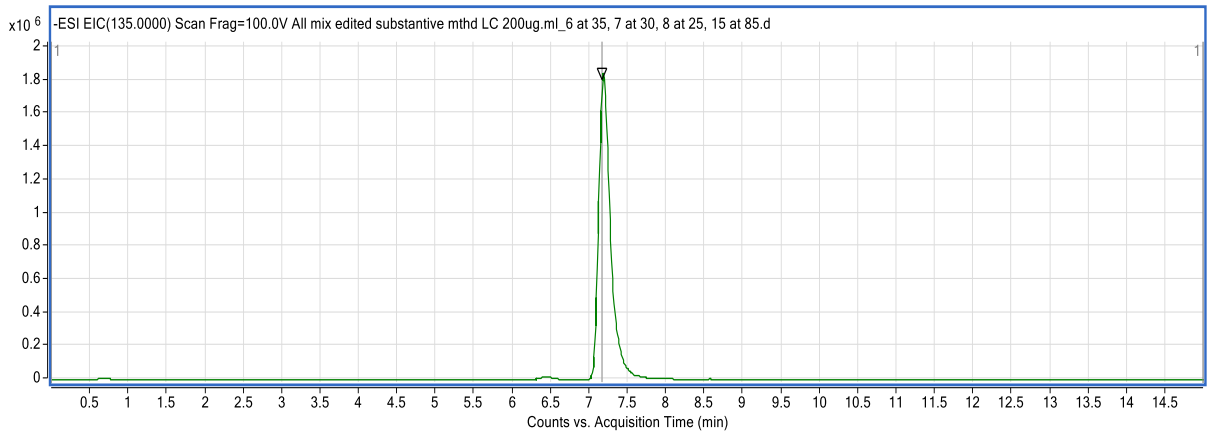
Time	A [H_2O] %	B [ACN] %
0	85	15
2	70	30
3	60	40
4	55	45
5	50	50
6	35	65
7	30	70
8	25	75
15	85	15

Best separation and best resolution

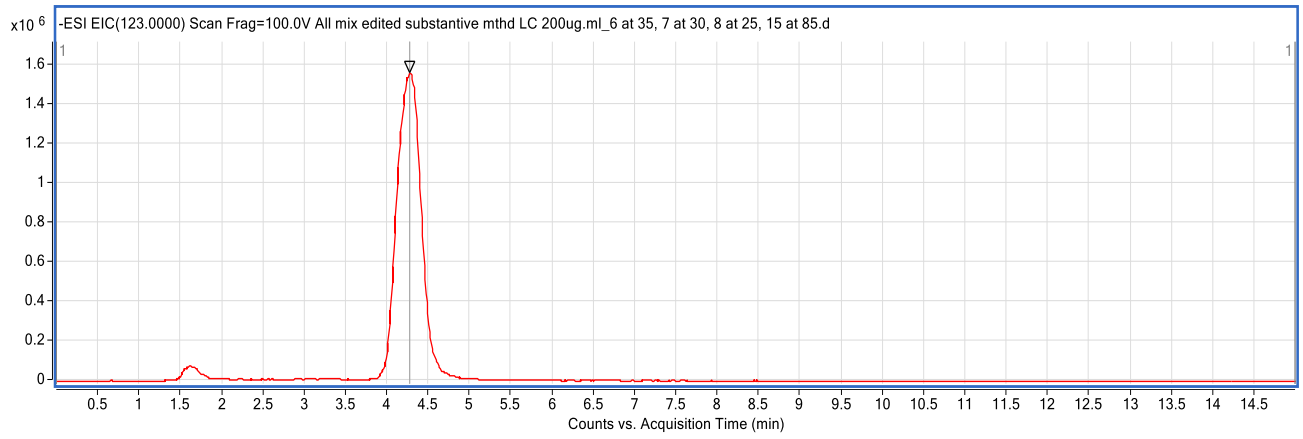




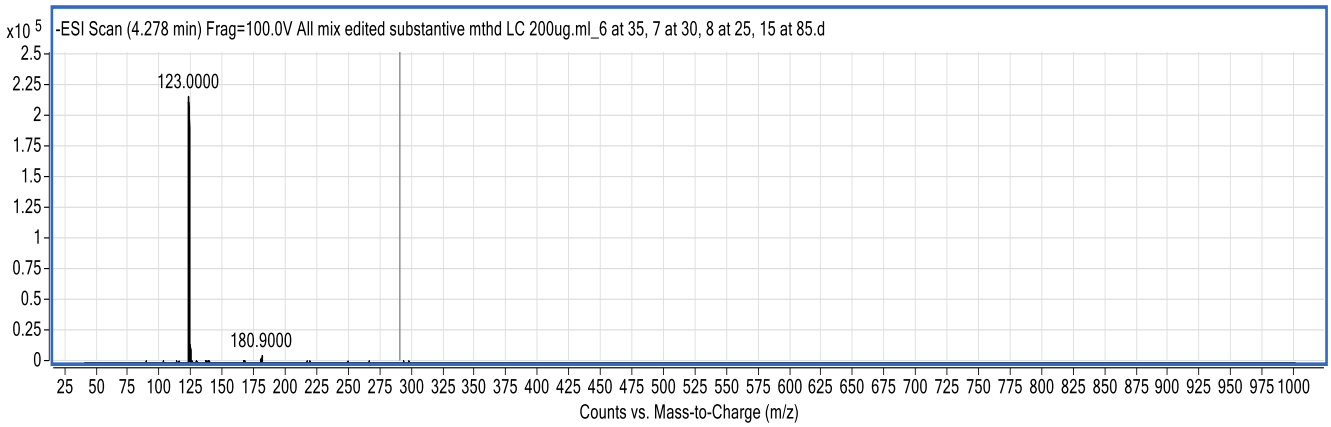
Overlaid chromatogram of the total ion chromatogram of M2Scan and the extracted ion chromatogram showing the ions of interest: Guaiacol (m/z 123), Methyl Benzoic acid (m/z 135), and Naphthoic acid (m/z171)respectively in negative polarity.



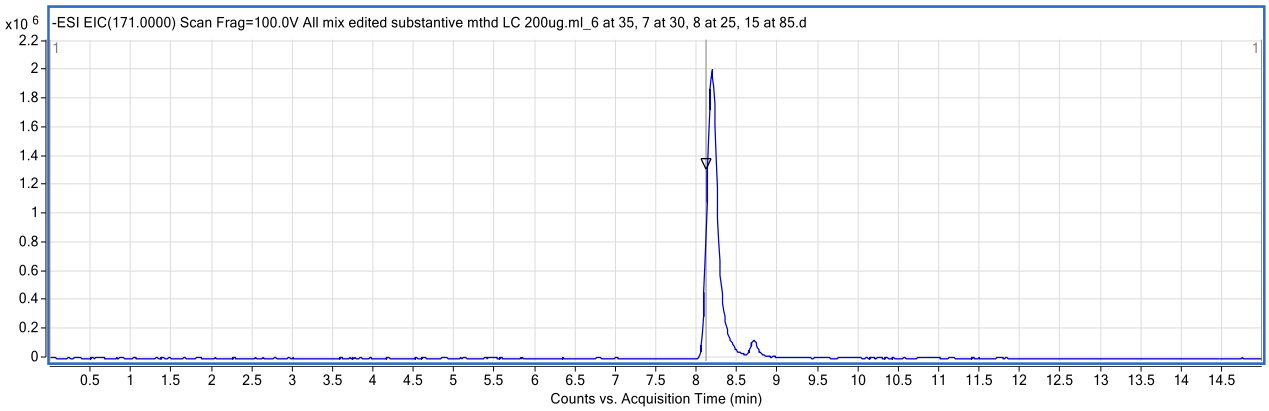
The chromatogram of the best gradient program showing methyl benzoic acid



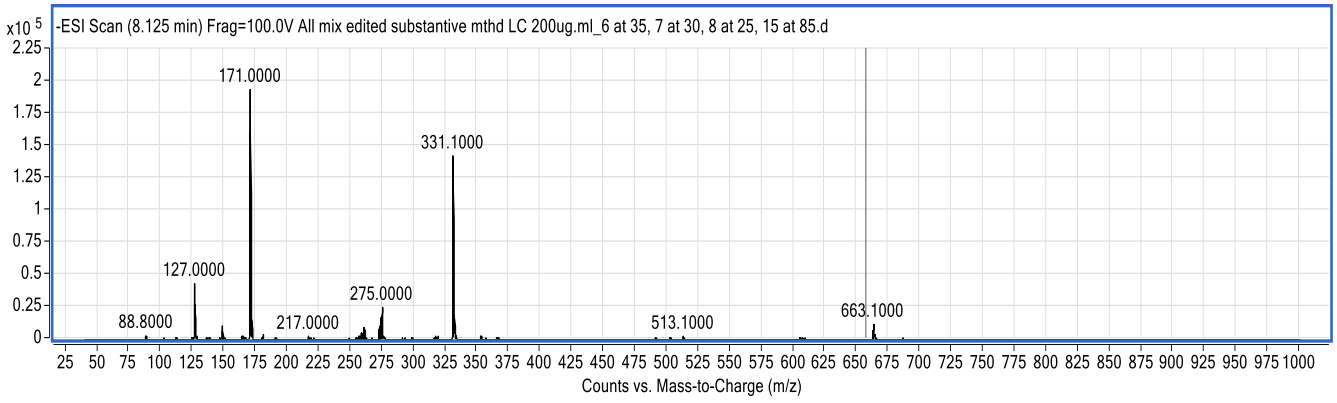
The chromatogram of the best gradient program showing guaiacol



The mass spectra of the best gradient program showing guaiacol (m/z 123)

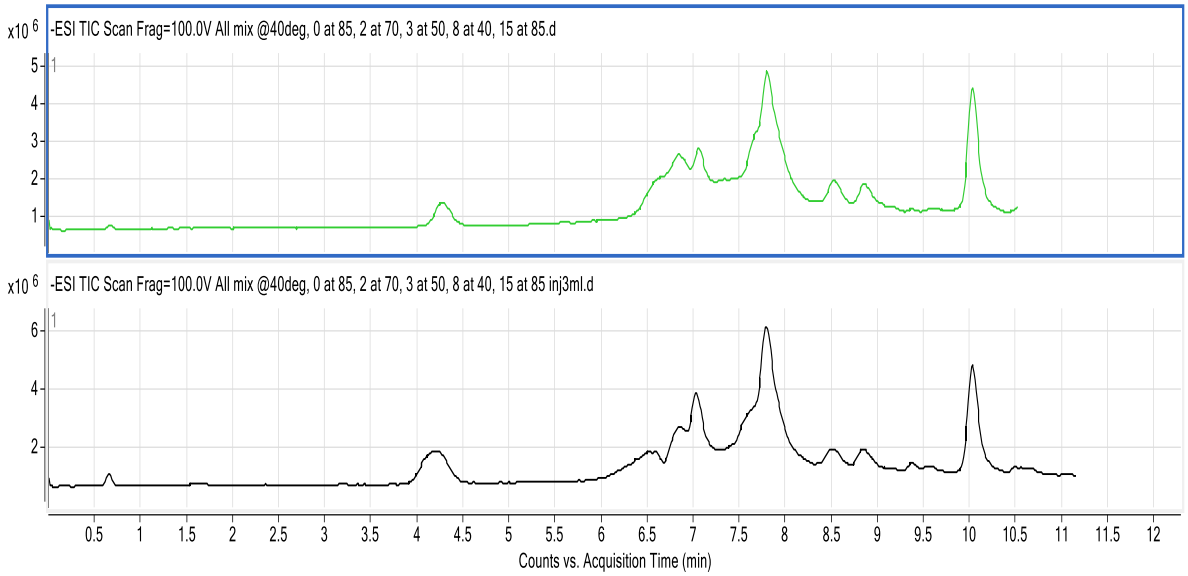


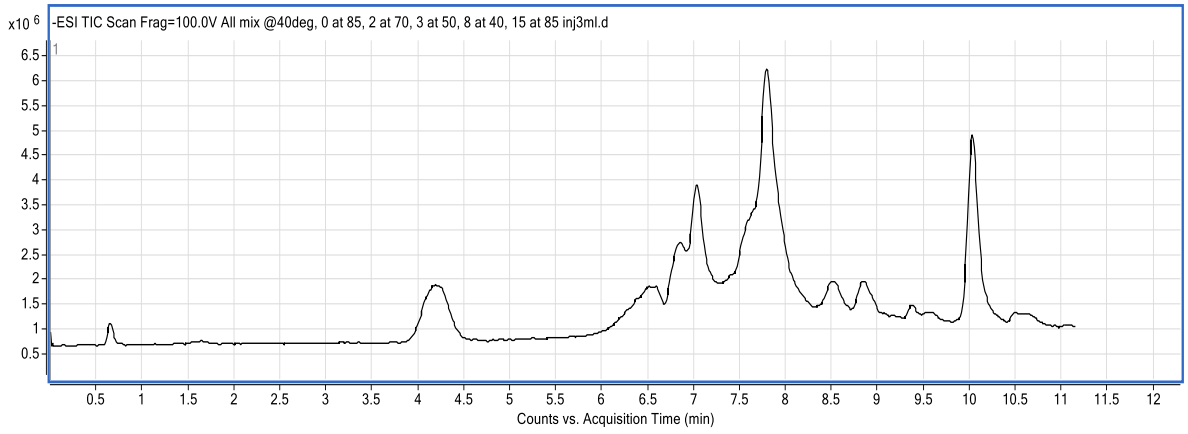
The chromatogram of the best gradient program showing Naphthoic acid

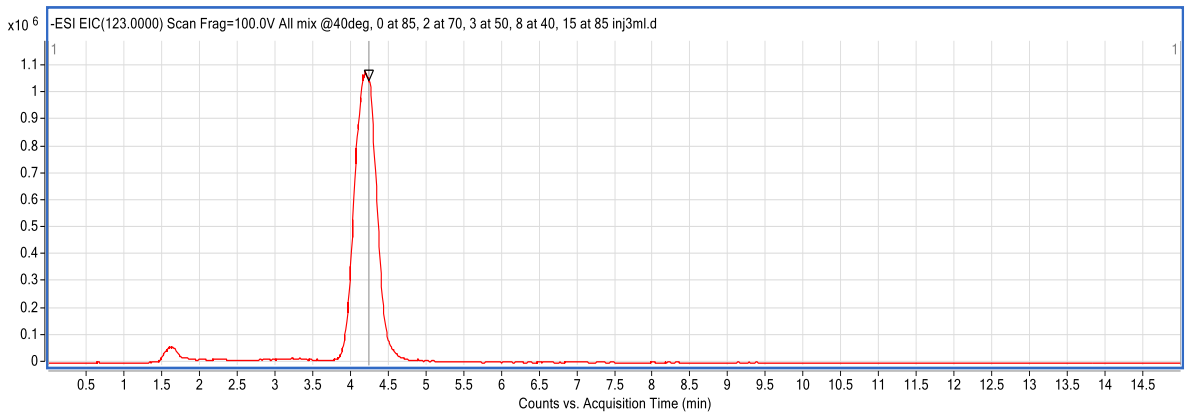


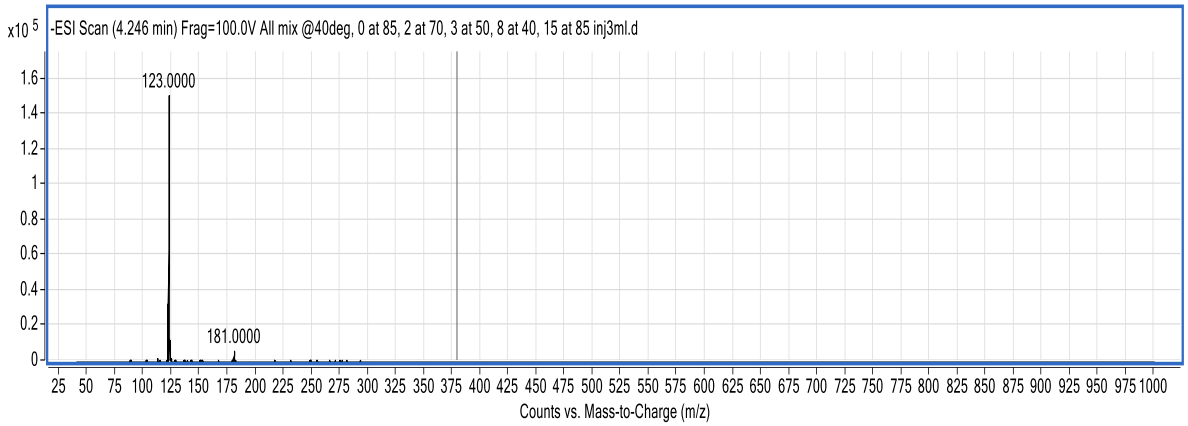
The spectra of the best gradient program showing Naphthoic acid (m/z 171) and the fragment of 127

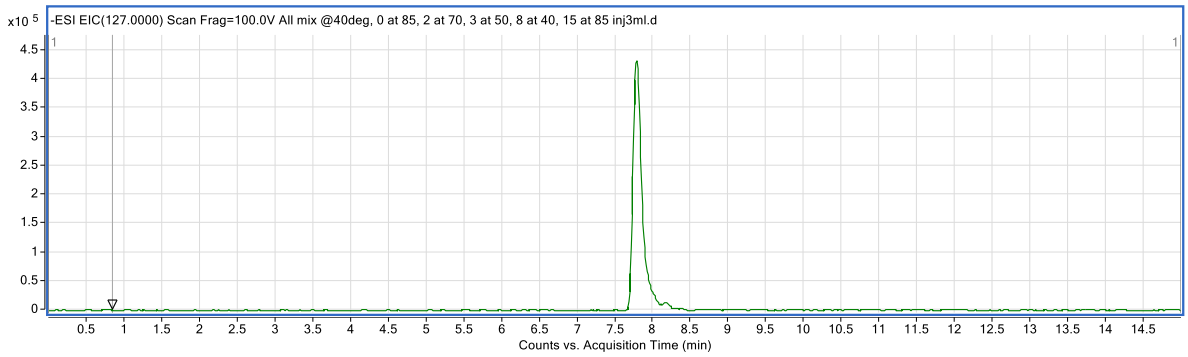
Time	A [H_2O] %	B [ACN] %
0	85	15
2	70	30
3	60	40
4	55	45
5	50	50
6	40	60
7	35	65
8	30	70
15	85	15

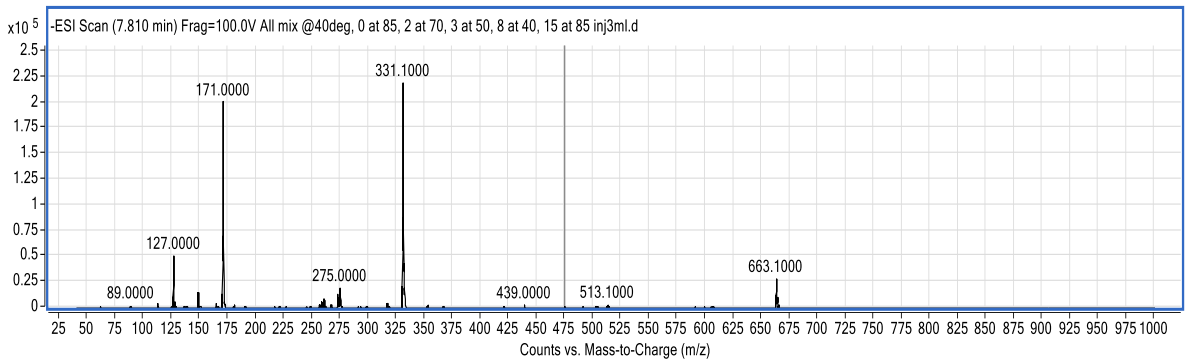


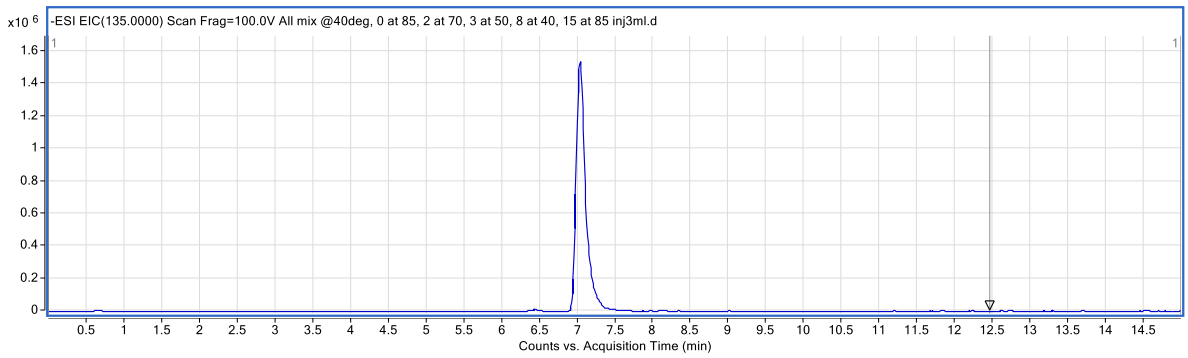


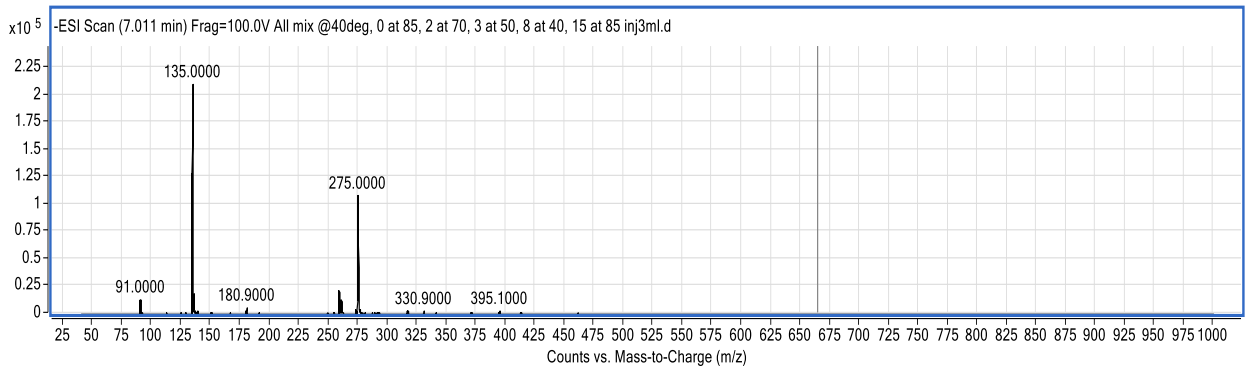

x10 6
-ESI EIC(123.0000) Scan Frag=100.0V All mix @40deg, 0 at 85, 2 at 70, 3 at 50, 8 at 40, 15 at 85 inj3ml.d
Counts vs. Acquisition Time (min)

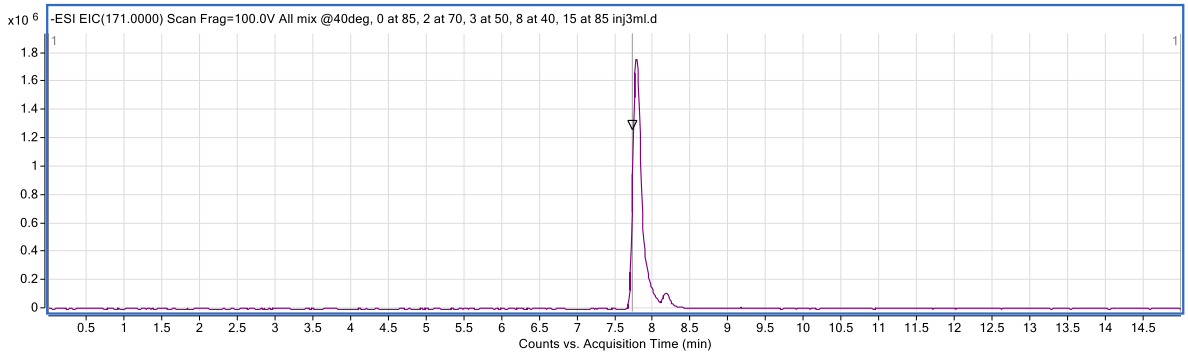

x10 5
-ESI Scan (4.246 min) Frag=100.0V All mix @40deg, 0 at 85, 2 at 70, 3 at 50, 8 at 40, 15 at 85 inj3ml.d
123.0000
181.0000
Counts vs. Mass-to-Charge (m/z)

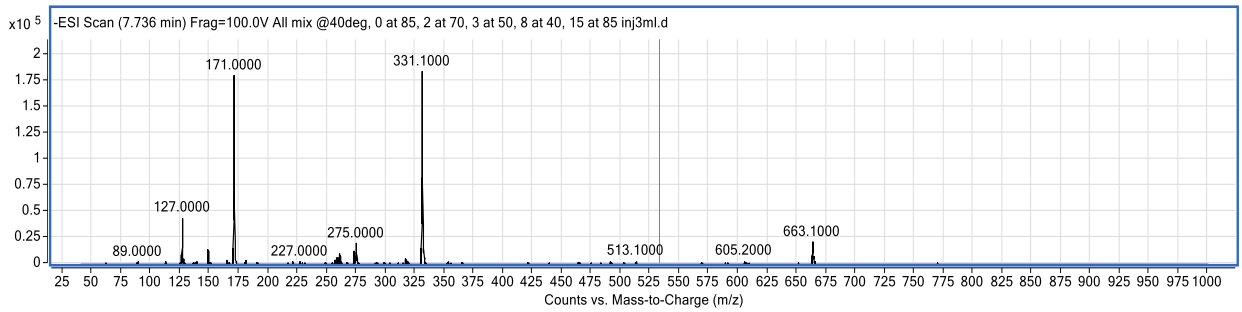

x10 5
-ESI EIC(127.0000) Scan Frag=100.0V All mix @40deg, 0 at 85, 2 at 70, 3 at 50, 8 at 40, 15 at 85 inj3ml.d
Counts vs. Acquisition Time (min)


x10 5
-ESI Scan (7.810 min) Frag=100.0V All mix @40deg, 0 at 85, 2 at 70, 3 at 50, 8 at 40, 15 at 85 inj3ml.d
331.1000
171.0000
127.0000
275.0000
89.0000
439.0000
513.1000
663.1000
Counts vs. Mass-to-Charge (m/z)

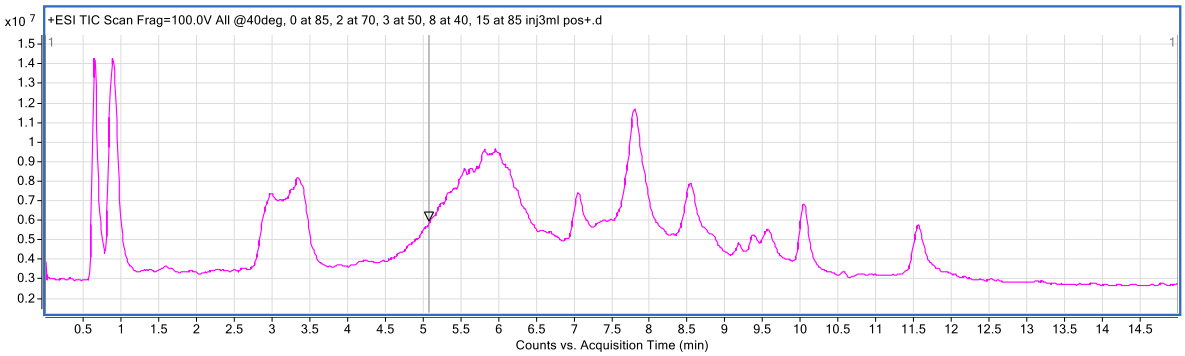

x10 6
-ESI EIC(135.0000) Scan Frag=100.0V All mix @40deg, 0 at 85, 2 at 70, 3 at 50, 8 at 40, 15 at 85 inj3ml.d
Counts vs. Acquisition Time (min)

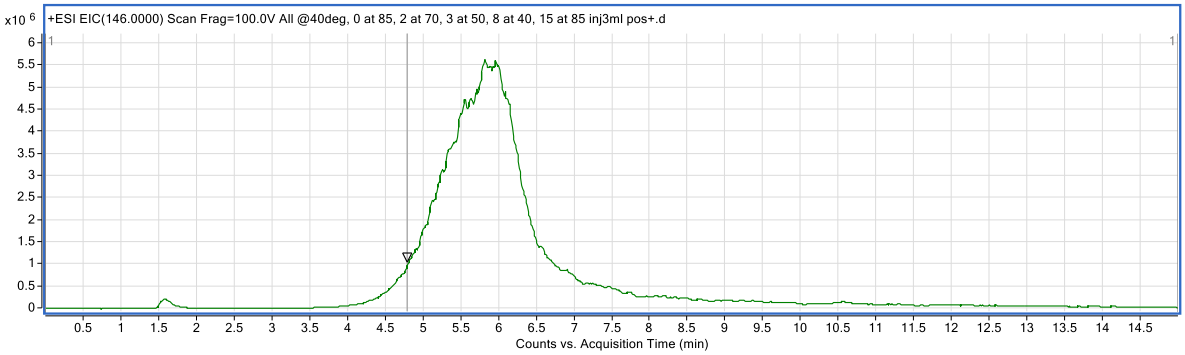

x10 5
-ESI Scan (7.011 min) Frag=100.0V All mix @40deg, 0 at 85, 2 at 70, 3 at 50, 8 at 40, 15 at 85 inj3ml.d
135.0000
275.0000
91.0000
180.9000
330.9000
395.1000
Counts vs. Mass-to-Charge (m/z)

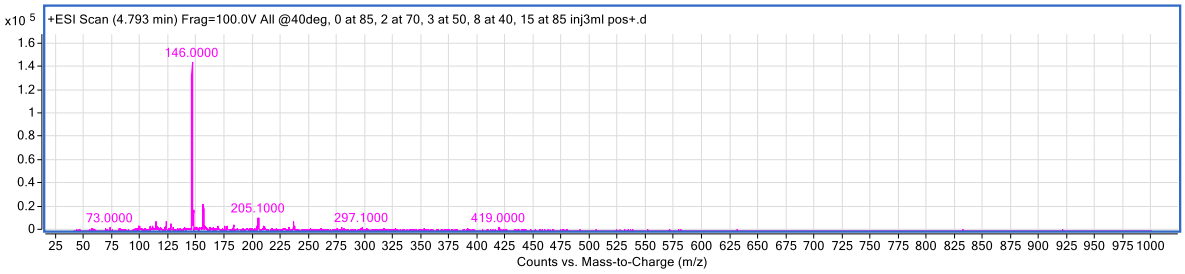

x10 6
-ESI EIC(171.0000) Scan Frag=100.0V All mix @40deg, 0 at 85, 2 at 70, 3 at 50, 8 at 40, 15 at 85 inj3ml.d
Counts vs. Acquisition Time (min)

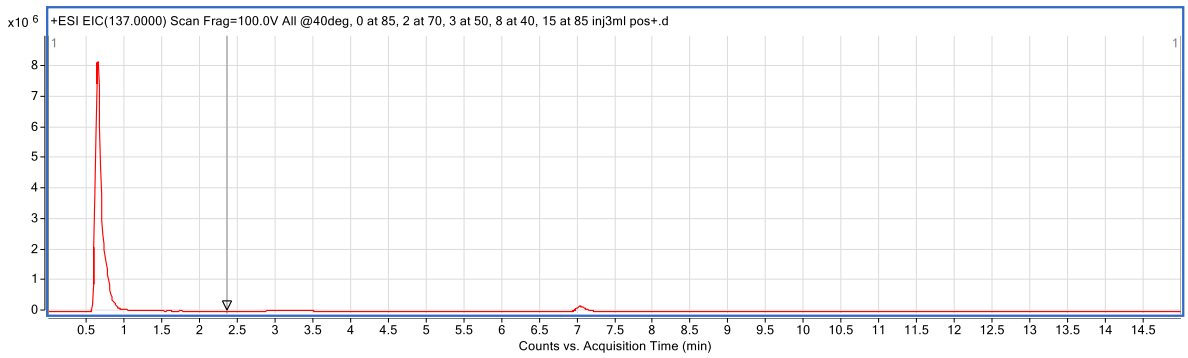

x10 5
-ESI Scan (7.736 min) Frag=100.0V All mix @40deg, 0 at 85, 2 at 70, 3 at 50, 8 at 40, 15 at 85 inj3ml.d
171.0000
331.1000
127.0000
275.0000
663.1000
89.0000
227.0000
513.1000
605.2000
Counts vs. Mass-to-Charge (m/z)

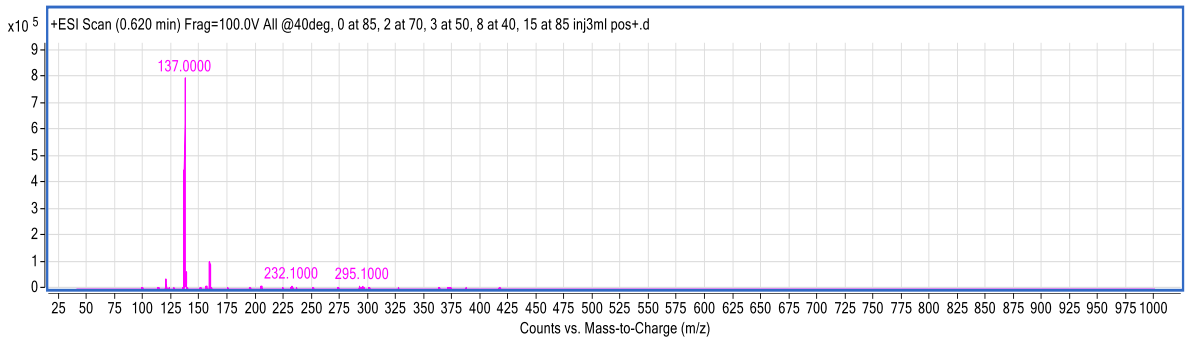
Positive polarity

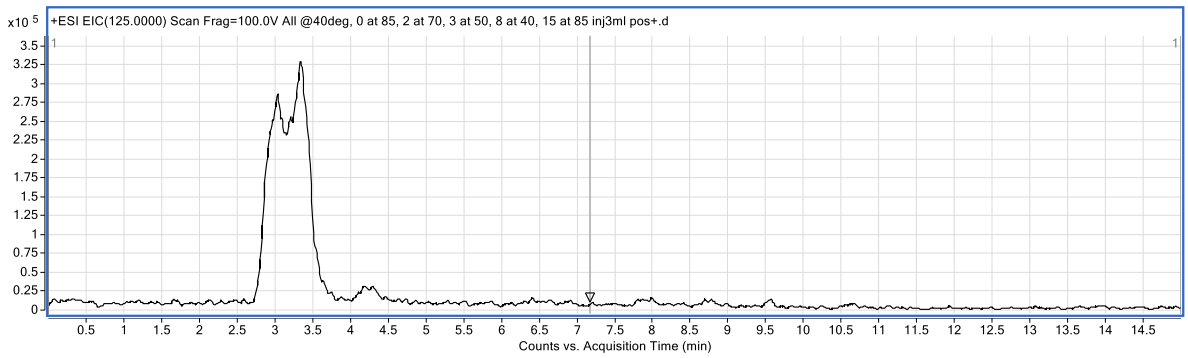


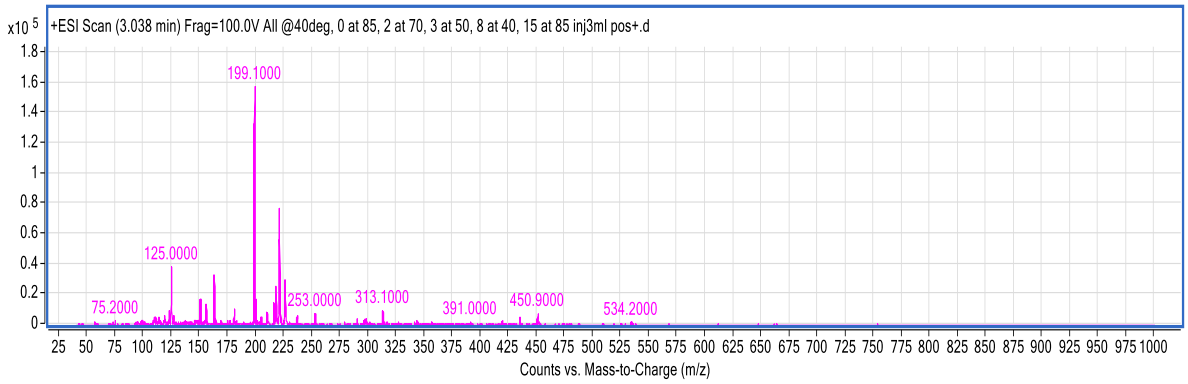





x10 6
+ESI EIC(137.0000) Scan Frag=100.0V All @40deg, 0 at 85, 2 at 70, 3 at 50, 8 at 40, 15 at 85 inj3ml pos+.d
Counts vs. Acquisition Time (min)


x10 5
+ESI Scan (0.620 min) Frag=100.0V All @40deg, 0 at 85, 2 at 70, 3 at 50, 8 at 40, 15 at 85 inj3ml pos+.d
137.0000
232.1000
295.1000
Counts vs. Mass-to-Charge (m/z)


x10 5
+ESI EIC(125.0000) Scan Frag=100.0V All @40deg, 0 at 85, 2 at 70, 3 at 50, 8 at 40, 15 at 85 inj3ml pos+.d
Counts vs. Acquisition Time (min)


x10 5
+ESI Scan (3.038 min) Frag=100.0V All @40deg, 0 at 85, 2 at 70, 3 at 50, 8 at 40, 15 at 85 inj3ml pos+.d
199.1000
125.0000
75.2000
253.0000
313.1000
391.0000
450.9000
534.2000
Counts vs. Mass-to-Charge (m/z)